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MODIFICATION OF THE ACTION OF CAFFEINE ON THE SPONTANEOUS ACTIVITY OF THE WHITE RAT BY THE CONCOMITANT ADMINISTRATION OF VARIOUS FOOD MATERIALS [1, 2]

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SEVEN FIGURES

(Received for publication June 23, 1947)

Various investigators have found that caffeine above a certain threshold level has a stimulating action on the central nervous system (Hollingworth, '12; Cheney, '35; Horst and Jenkins, '35).

The present experiments are offered to show that this action of caffeine as manifested by an increase in the spontaneous activity of the albino rat, can be modified to an appreciable extent by certain food materials administered either simultaneously with the administration of caffeine or a short time afterward.

PROCEDURE

The method used for measuring spontaneous activity was that described by Schulte and his associates ('41). The apparatus consisted of a small wire cage measuring 6″ × 6″ × 6″ which was suspended from a spiral spring in such a manner

[1] Preliminary report, Fed. Proc., *6*: 119, 1947.

[2] The expense of this investigation was defrayed in large part by a grant-in-aid from the Sugar Research Foundation.

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in the activity cages for the first time. They were kept in the cages for 1 hour for the purpose of obtaining their basal activity, then removed and given caffeine or water by stomach tube and replaced in the cages. This procedure was repeated on successive days (except on Sundays) until 220 daily observations had been made on the test animals and the same number on the controls.

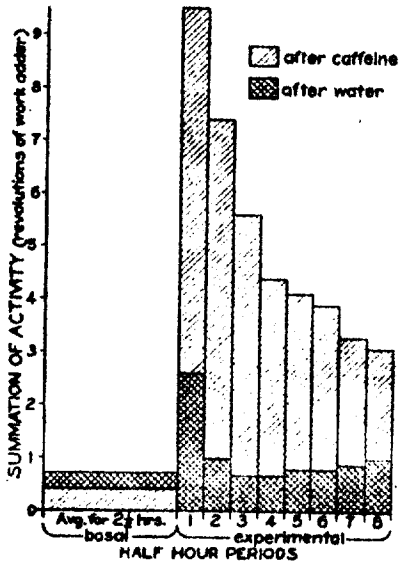

Figure 1

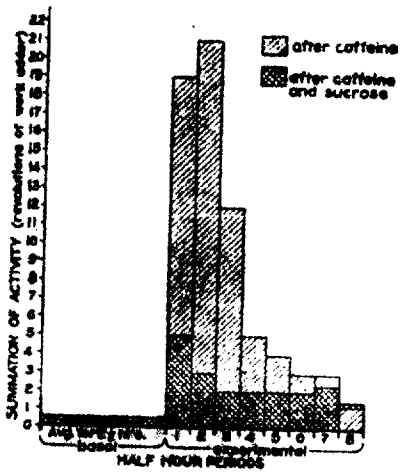

Figure 2

Fig. 1 The effect of 75 mg caffeine/kg on spontaneous activity. For the sake of continuity the activity of the animals under test and control conditions is represented by hatched and cross-hatched lines, respectively, although caffeine was not given to the test animals nor water to the controls until the end of the basal period.

Fig. 2 Comparison of spontaneous activity after caffeine in water and caffeine in a sucrose solution.

Seventy-five mg caffeine per kg body weight had a definite stimulating effect on spontaneous activity which was most marked during the first half hour. This effect was still manifest at the conclusion of the experiment 4 hours after the administration of caffeine. The results of the experiment are presented in figure 1. The activity of the test animals was

that all the movements of the animals, even the slightest ones, were summated by a work adder attached to the spring. A small pin inserted in the rim of the work adder dipped into a mercury cup upon each complete revolution thereby completing an electric circuit and activating a signal magnet writing on a slowly moving kymograph paper. Activity was measured and recorded in terms of the total number of revolutions of the work adder throughout the experiment. Twelve units which were set up in a small well-lighted room devoted exclusively at the time to the experiment were used simultaneously. All the experiments were run in the morning because at this time of day in a well-lighted room the activity of a white rat is at a minimum.

Seven sets of observations were made under different experimental conditions. Caffeine alkaloid in various amounts was administered in aqueous solution by stomach tube or intraperitoneally. In some experiments sugar dissolved in water was given by stomach tube simultaneously with or shortly after the administration of caffeine. In others peptone, vegetable oil, and the inert material agar were given instead of sugar. Further details of the procedure will be described under the following separate captions.

RESULTS

Experiment 1: Showing that caffeine causes an increase in spontaneous activity for several hours after administration whereas no permanent effect is produced by the daily consumption of a large amount of caffeine

Twenty-two animals were selected 1 week after weaning and given 75 mg caffeine/kilo daily in aqueous solution by stomach tube for 100 days. An equal number of litter mates were chosen for controls and by the same procedure given water daily instead of the caffeine solution. At the end of this period 24 hours were allowed to elapse without the administration of caffeine whereupon the animals were placed

greater than that of the controls in 206 out of 220 individual experiments; in 5 experiments there was difference whereas in 9, the controls were slightly more active than the test animals.

Long continued daily administration of this amount of caffeine had no permanent effect as shown by the fact that the basal activity of the animals that had been given caffeine for 100 days was the same as that of the controls. In this connection it is of interest to note that in human subjects Cheney ('35) found no significant effect on reaction time 24 hours after the consumption of caffeine.

Experiment 2: Showing that sucrose administered simultaneously with caffeine reduces the increase in spontaneous activity caused by the stimulating action of caffeine

These experiments were undertaken with the purpose in mind of following up a lead obtained in other observations (not published) which suggested that the action of caffeine might be affected by the simultaneous ingestion of sugar.

In this and the following experiments each animal served as its own control. The animals were divided into 2 groups. On the first day of the experiment 1 group was studied under experimental and the other under control conditions. On alternate days thereafter this order was reversed.

Twelve adult rats were selected at random from our colony. Under control conditions the animals were given 20 mg caffeine/kg in aqueous solution by stomach tube and under test conditions the same amount of caffeine in a 50% sucrose solution (1.5 gm sucrose/100 gm body weight). It had been found previously in another set of experiments that 20 mg caffeine/kg had a definite stimulating action. These results are not presented separately as they are in agreement with those obtained with this amount of caffeine in the experiments shown in figure 2. Each point on the nomogram is plotted from an average of 60 experiments.

plements of caffeine, theobromine or theophylline. It was observed that all three compounds were lipotropic.

Experimental

Male weanling rats of the Sprague-Dawley strain were fed a diet of the following composition: purified casein,[1] 6.0; hydrogenated cottonseed oil, [2] 38.0; U.S.P. cod liver oil, 5.0; salts,[3] 2.0; and cane sugar, 49.0. The diet also contained in mg./kg.: thiamine hydrochloride, 10; calcium pantothenate, 40; nicotinic acid, 40; pyridoxine

TABLE I

Influence of Caffeine, Theobromine and Theophylline on Liver Fat Content of Rats Fed Choline-Deficient Diet

Group	No. of rats	Dietary treatment	Initial body weight (average)	Final body weight (average)	Liver weight (average)	Liver fat content (average)
		Experiment 1				
			g.	*g.*	*g.*	*per cent*
I	10	Basal diet for 21 days	46.5±1.4[a]	45.5±1.7	4.12±0.22	28.4±1.5
II	8	Basal diet for 27 days	45.6±1.1	46.3±2.0	4.02±0.28	29.3±2.9
III	13	0.3% Caffeine for 21 days	48.1±1.6	39.6±1.8	2.24±0.09	11.9±1.1
IV	11	0.3% Caffeine for 27 days	49.9±1.7	40.4±1.5	2.66±0.16	17.3±1.8
V	7	0.3% Caffeine for 13 days, then 0.4% caffeine for 8 days.	45.9±2.2	36.1±2.0	2.04±0.09	9.5±1.0
VI	5	0.3% Caffeine for 13 days, then 0.4% caffeine for 8 days, then 0.6% caffeine for 7 days.	46.0±1.7	34.6±1.7	2.16±0.13	13.5±2.3
		Experiment 2				
VII	10	Basal diet for 26 days	35.4±1.1	38.6±1.1	3.93±0.23	32.3±2.2
VIII	10	0.4% Theobromine 16 days, then 0.6% theobromine for 10 days.	35.9±0.9	32.0±1.3	2.11±0.31	12.4±1.1
IX	8[b]	0.4% Theophylline for 26 days.	34.6±1.5	27.4±1.4	1.14±0.26	12.6±1.6

[a] Including the standard error of the mean calculated as $\sqrt{d^2/N-1}/\sqrt{N}$.
[b] 10 Rats were begun on experiment of which 2 died after 6 and 7 days.

[1] General Brands, Inc.
[2] Crisco.
[3] Hubbell, Mendel and Wakeman (6).

hydrochloride, 10; riboflavin, 20; biotin, 0.01; 2-methyl-1,4-naphthoquinone, 4. Once weekly the rats received 3 mg. of α-tocopherol dissolved in 0.03 ml. ethyl laurate. Some of the animals obtained supplements of caffeine, theobromine or theophylline mixed in their diet. The compounds were commercial preparations that had been recrystallized. The rats were kept in individual metabolism cages.

In Expt. 1 (Table I) the rats were divided into 6 groups. Group I received the basal diet for 21 days, after which the rats were killed and their livers analyzed for total fat. Group II was fed the basal diet for 27 days. Groups III and IV received 0.3% caffeine in their diet for 21 and 27 days respectively. The diet of group V contained 0.3% caffeine during the first 13 days on test. The rats were then given 0.4% caffeine for 8 days. Group VI received 0.3% caffeine in the diet for 13 days, then 0.4% caffeine for 8 days and finally 0.6% caffeine for 7 days. The level was increased stepwise in the hope that the rats would develop some tolerance to the drug. Fifteen litters of rats were used for the experiment. One member of each litter was placed on the basal ration while the others received the supplemented diets.

In Expt. 2 (Table I) 30 rats were divided into 3 groups, equal with respect to litter distribution. Group VII was fed the basal choline-deficient diet for 26 days. Group VIII received a dietary supplement of 0.4% theobromine for 16 days and 0.6% during the next 10 days. Group IX was fed 0.4% theophylline for 26 days.

The livers were weighed and analyzed for total fat by the method of Artom and Fishman (7). This involves preliminary extraction with a mixture of hot alcohol and ether. After evaporation nearly to dryness the alcohol-ether extractable material is reextracted with hot chloroform and finally dried and weighed.

Results

The content of total chloroform-soluble material in the livers of the rats is shown in Table I. The differences in liver fat content between the groups on the basal diet and those fed supplements were analyzed by Fisher's t-method (8) and found to be significant ($p < 0.01$). In the present study no comparison was made between the lipotropic activity of the methylated purines and methionine. But in connection with another investigation 20 male weanling rats of the same strain were kept for 23 days on the basal diet described in this paper supplemented with 1% methionine. This is equivalent in total methyl content to 0.47% caffeine. The rats ate an average of 2.6 g./day and their liver fat content was 10.5 ± 0.9%. Eighteen unsupplemented rats also ate 2.6 g./day and showed a liver fat content of 38.9 ± 0.7%.

Table II gives the average daily food consumption for the rats in the present study. It is evident that food intake was much poorer for the group fed theophylline than for the others. The rats given theophylline appeared nervous and restless. Two of them died, after 6 and 7 days on the diet. The animals fed the other two supplements showed no evidence of intoxication, except for loss in weight.

TABLE II

Average Daily Food Consumption of Young Rats on a Basal Choline-Deficient Diet and with Various Supplements Added to the Diet

Supplement	Content in diet	Average daily food consumption
	Experiment 1	
	per cent	g.
None	—	2.9±0.1
Caffeine	0.3	2.6±0.1
Caffeine	0.4	2.6±0.5
Caffeine	0.6	2.5±0.3
	Experiment 2	
None	—	2.5±0.1
Theobromine	0.4	2.8±0.2
Theobromine	0.6	2.6±0.1
Theophylline	0.4	1.8±0.1

Discussion

These experiments show that caffeine, theophylline and theobromine have lipotropic activity. This is presumably due to the fact that these compounds can be demethylated and make possible choline synthesis in the rat. However, it is conceivable that some peculiar pharmacological property of the compounds may be responsible for the effect observed. Thus, Hindemith (9) has stated that caffeine increased the unsaturation of liver fatty acids.

In a single feeding trial we confirmed Moyer and du Vigneaud (4), who found that caffeine did not stimulate growth when added to a methionine- and choline-free diet containing homocystine. Another instance of a compound which prevented fatty livers and hemorrhagic kidneys in rats, and yet was relatively inefficient in substituting for choline on methyl-free diets, was dimethylaminoethanol (10).

Acknowledgment

Miss Louise Odor assisted in carrying out some of these experiments.

Summary

A low casein-high fat diet, deficient in choline, was fed to male weanling rats for several weeks, Some of the animals received supplements of caffeine, theobromine or theophylline. The content of these methylated purines in the diet varied from 0.3 to 0.6%. At the con-

clusion of the feeding period the animals were killed and their livers were analyzed for total chloroform-soluble substances. All three compounds were found to be lipotropic.

References

1. Cushny's Pharmacology and Therapeutics. 12th ed., revised by C. W. Edmunds and J. A. Gunn. Lea and Febiger, Philadelphia, 1940.
2. Myers, V. C., and Hanzal, R. F., *J. Biol. Chem.* **162**, 309 (1946).
3. Bernheim, F., and Bernheim, M. L. C., *Arch. Biochem.* **12**, 249 (1947).
4. Moyer, A. W., and du Vigneaud, V., *J. Biol. Chem.* **143**, 373 (1942).
5. Mills, C. A., and Cottingham, E., *Arch. Biochem.* **4**, 171 (1944).
6. Hubbell, R. B., Mendel, L. B., and Wakeman, A. J., *J. Nutrition* **14**, 273 (1937).
7. Artom, C., and Fishman, W. H., *J. Biol. Chem.* **148**, 405 (1943). Additional details supplied in a personal communication.
8. Fisher, R. A., Statistical Methods for Research Workers. Oliver and Boyd, London, 1936.
9. Hindemith, H., *Arch. exptl. Path. Pharmakol.* **199**, 167 (1942).
10. du Vigneaud, V., Chandler, J. P., Simmonds, S., Moyer, A. W., and Cohen, M., *J. Biol. Chem.* **164**, 603 (1946).

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Does Coffee Influence the Lipid Metabolism?

By S. Heyden

With 1 figure and 3 tables

(Received June 16, 1969)

It is desirable to divide this topic into two sections, the first one dealing with the influence of caffeine on free fatty acids, the second one with the influence on cholesterol metabolism and alleged consequences of atherogenesis.

a) Influence of Caffeine on Free Fatty Acids

A considerable amount of literature has accumulated in the last five years pertaining to the rise of plasma free fatty acid concentrations after ingestion of coffee, intravenous and intramuscular caffeine injections and after drinking coca cola (Bellet et al. 1968) which contains caffeine. There are wide individual variations in the response of the free fatty acid levels to caffeine; however, in general a significant increase over control levels *within* four hours after administration of caffeine can be observed. The peak free fatty acid concentration in the blood of dogs after caffeine administration occurs two hours later. In human subjects the peak concentration of free fatty acids is given at three to four hours after coffee ingestion.

An interesting finding made during these experiments was that glucose apparently blocked the free fatty acid increase normally produced by coffee ingestion. The suppression of caffeine-induced free fatty acid mobilization by sucrose or glucose is probably a result of the effect of sucrose and glucose on reesterification of the released free fatty acids. When the effect of glucose administration has dissipated, the lipolytic effect of caffeine is no longer counteracted by the glucose and a significant increase in plasma free fatty acids is observed (Bellet, Kershbaum and Finck 1968).

Although the measurement of blood caffeine concentrations has been plagued by methodological difficulties, it can be assumed that maximum concentrations of caffeine in the blood are reached within one hour, after which a gradual decline occurs over a period of seven to nine hours (Bellet et al. 1968).

There is nothing specific about the free fatty acid elevation induced by and associated with the use of coffee. Physical exercise, cigarette smoking, emotional stress, prolonged starvation and alcohol consumption in excessive amounts are all known to increase free fatty acids temporarily to a significant degree. The mobilization of free fatty acids in physical exercise, cigarette smoking and emotional stress is facilitated through circulating catecholamines. To produce an effective level of circulation catecholamines in the blood, both the adrenal glands and the sympathetic nervous system must be functioning concomitantly.

"There is little doubt that norepinephrine plays a cardinal role in elevating the FFA level during exercise" (RODAHL et al. 1964). Nobody has shown an *increase* in cholesterol levels following exercise, however. In cigarette smoking, KERSHBAUM and co-workers (1962, 1963) have demonstrated that the increased release of epinephrine and norepinephrine by the sympathetic nerve endings and adrenal glands cause the mobilization of free fatty acids from adipose tissues since in patients with bilateral adrenalectomy no free fatty acid elevation in the blood was seen after cigarette smoking. It was this group of investigators in Philadelphia, KERSHBAUM and BELLET, who had repeatedly stated that the free fatty acid elevation after cigarette smoking "may be important in view of subsequent hypercholesterolemia". However, during the last ten years it has become increasingly clear, mainly from epidemiologic studies, that cigarette smokers do not differ from non-cigarette smokers in regard to their cholesterol levels. The pooled data from the Albany and Framingham Heart Studies indicated that lipid levels are not correlated with smoking habits (DOYLE 1968). BLACKBURN and co-workers (1960) did not find any association between cigarette smoking and higher cholesterol levels in a long-term study.

Table 1. Cholesterol Levels in Non-smokers and Smokers

Occupational Groups	Non-Smokers	Cigarette Smokers
Business Men	234.8 mg%	242.7 mg%
Students	173.7 mg%	179.0 mg%
Railroad Workers	224.8 mg%	232.2 mg%
Firemen	241.7 mg%	251.1 mg%

From: BLACKBURN et al., 1960.

In a cross-sectional study of 500 Swiss factory workers (males 30 to 60 years of age) cigarette smokers and their triglyceride and cholesterol levels were compared with the respective levels of "non-smokers", including ex-smokers, cigar and pipe smokers and those who did not inhale cigarette smoke (HEYDEN 1967). The differences were insignificant: 23% of the cigarette smokers and 20% of the "non-smokers" had hypertriglyceridemia ($\geq$ 135 mg%). Hypercholesterolemia ($\geq$ 250 mg%) was equally seen in 47% of cigarette smokers and 47% of "non-smokers". On the basis of these findings we cannot subscribe to a visible long-term effect of nicotine on blood lipids. EPSTEIN (1965) likewise stated "the effect is probably not mediated by cholesterol (or blood pressure) since these variables are not consistently associated with smoking".

The other frequent assumption that cholesterol levels are increased under conditions of emotional stress has been refuted in several studies. LEVI (1967) in a very careful short term experiment on 33 male volunteers has documented a significant increase of free fatty acids in the serum of men undergoing stress situations whereas the cholesterol levels in the same subjects remained unchanged. Similarly, RAAB and KRZYWANEK (1966) found mean FFA levels markedly elevated during combined sensory and mental stresses but serum

cholesterol concentrations were not influenced. Although it has become a popular opinion that cholesterol levels rise with emotional stress, more recent investigations have shown that there was really no difference in cholesterol levels of men undergoing stressful situations or being in stressful occupations (Lit. see HEYDEN 1969).

Finally, the effect of prolonged starvation on blood lipids of obese subjects is usually a fall of cholesterol and triglyceride levels, but a rise of FFA levels above baseline (JACKSON 1969).

b) Influence of Caffeine on Cholesterol Metabolism

To return to the problem of cholesterol metabolism and coffee consumption we are faced with two studies in human subjects which contradict each other but nevertheless have caused great concern to laymen and physicians alike.

1. LITTLE and co-workers (1965) tried to correlate dietary intake with serum cholesterol levels in 86 patients with coronary heart disease and 84 control persons and found none of the correlation coefficients statistically significant. Among the dietary factors considered were coffee and tea consumption, as indicated by the number of cups of coffee, the number of cups of tea and the number of cups of tea and coffee combined.

They stated: "Our coronary patients took less tea and coffee and less sugar than the control persons". However, their coronary group when examined alone showed a positive and statistically significant correlation of coffee intake with *plasma cholesterol*. In 1966 the same group (LITTLE et al.) reported that in the coronary group coffee showed a consistently positive and significant correlation with each of the serum lipid and serum lipoprotein fractions measured. The authors mentioned that "in our retrospective study of Canadians, coronary patients and controls drink equal amounts of coffee. This difference in results (in comparison to the Americans examined by Paul) cannot be explained unless after the onset of coronary symptoms patients drank less coffee". And later they also stated, "The data suggest that coffee contains a substance which elevates serum lipids in susceptible persons and that such persons may be liable to coronary heart disease. It has yet to be shown, however, that other serum lipid fractions (besides plasma unesterified fatty acids) will become elevated with long-term drinking of coffee or that increased atherosclerosis results".

2. The second investigation, an epidemiologic study by PAUL and co-workers (1963) found that there was a significant correlation between the use of coffee and the later development of coronary disease ($P < 0.025$). In 1968 this same group reported: "We did not have evidence that the serum cholesterol level was significantly higher in the group with the highest coffee consumption, although it was slightly higher in these men than in those with the lowest or no coffee consumption. It did not appear that the association of coffee drinking with an increased incidence of coronary disease could be entirely explained on this basis".

We have voiced our criticism that although there was statistical significance it was really not enough to make one confident of the biological importance. The statistical significance in this first analysis back in 1963 depended on the excessive coffee intake of 10 patients out of 54 coronary

patients. Even if taken at face value, the effect on the coronary arteries could not necessarily be attributed to the coffee intake because of the competing effect of other factors, e. g. nicotine inhalation which had not been taken into account in this particular statement by PAUL. It is a general observation that those individuals who are excessive coffee drinkers and also smoke tend to smoke cigarettes excessively. In a second study by PAUL and co-workers reported in the Lancet of November, 1968, these factors were considered together and it was now stated that the simple relation between the coffee and cigarette habit was analyzed and they are significantly associated with a $P < 0.001$. An additional, more statistically sophisticated, analysis of the 1963 data was performed. The 66 subjects with CHD historics were compared to 85 randomly selected controls using the logit transformation. This transformation allows for the comparison of the risks of high and low coffee drinkers separately from the risks attendent on cigarette smoking. The results were summarized: "It is clear that the high cigarette users are severely at risk. The heavy coffee drinkers have somewhat more myocardial infarctions and deaths from coronary disease than do those who take little, but this difference is not statistically significant". The important point about this analysis is that the transformation has eliminated the interaction between cigarette smoking and coffee drinking. The authors concluded by stating "The heavy use of coffee may be implicated as a risk factor although the importance is clearly much less (than cigarettes) and may be questioned".

It is evident, then, that the alleged statistically significant correlation between coffee consumption and coronary heart disease development may even be biologically unimportant. Neither a study by WALKER and GREGORATOS (1967) at the Walter Reed Army Hospital in Washington nor the prospective epidemiological study in Framingham, Massachusetts (KANNEL 1966) has shown a significant association between the excessive use of coffee and the development of coronary heart disease.

It is interesting to note that ZELLER and AMMON were unable to increase the cholesterol level or the level of esterified fatty acids in the serum of 52 patients with liver disease as well as 41 healthy control persons after injections of caffeine or drinking of coffee. These results were obtained in six laboratory determinations on each subject over a period of three hours after intravenous application of 250 mg of caffeine or consumption of 225 mg of caffeine. However, as expected, they saw a marked increment of free fatty acids in healthy persons with a peak serum level two hours after caffeine administration and a peak serum level one to six hours after caffeine administration in patients with liver disease.

Two British studies, one by BROWN in 1962 and the other one by NAISMITH and co-workers in 1969, could not show a positive correlation between use of coffee and coronary heart disease or coffee consumption and cholesterol levels. The study by NAISMITH and co-workers which was just reported was conducted on 20 healthy adult volunteers with an average age of 32. From records of the number of cups of tea and coffee consumed for ten consecutive days, the 'background' daily intake of caffeine for the individual and for the group was calculated. During the first two weeks of the experiment each subject drank ten cups of decaffeinated coffee per day totaling 20 gm Sanka instant coffee containing 12 mg caffeine. Subsequently, for 20 days 10 cups of coffee (20 gm

Maxwell House instant coffee = 875 mg caffeine) were consumed daily. The subjects were asked not to make any changes in their customary diets apart from avoiding other beverages containing caffeine (Fig. 1).

The mean 'background' caffeine intake for the entire group was high (560 mg per day). After two weeks on an almost *caffeine free regimen* plasma cholesterol levels were increased. The change in cholesterol levels was regarded statistically highly significant. The P value was not given. With the reintroduction of caffeine into the diet, the picture was reversed. The plasma cholesterol fell to near normal values and again the changes in cholesterol levels were given as statistically highly significant.

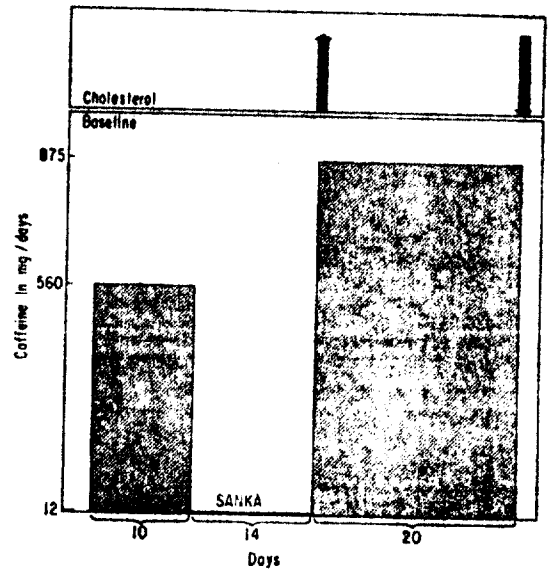

Fig. 1

Although the total impact of the previously mentioned observations in humans was that of essentially no correlation between coffee drinking and the development of hypercholesterolemia or ischemic heart disease, the suspicion had been raised and one solution seemed to be animal experimentation. Here again, the several reported results were negative, i.e. indicating a non-cholesterol elevating effect and non-atherogenic effect of caffeine. However, a few studies demonstrated the development of hypercholesterolemia, e. g. NAISMITH et al. 1969, the same group of investigators who found no cholesterol enhancing effect of caffeine in humans but rather a cholesterol lowering effect!

The hypothesis that coffee or caffeine might be harmful to the coronary arteries has been advanced most recently by a group of the Bio-Medical Division of the University of California. They contended "that the overt coffee drinking population has a higher degree of atherosclerosis than the overt tea drinking population" (YOUNG et al 1967).

They compared the degree of coronary atherosclerosis of 155 Caucasians whom they designated as 'coffee drinking population' with the degree of coronary atherosclerosis of 50 Chinese whom they designated as 'tea drinking population'. The age and sex were not mentioned (!). The mean degree of coronary atherosclerosis in Caucasians was given as "58", in the Chinese as "37". In view of the great variation with their method it is highly unlikely that these differences are significant. It is impossible to perform any statistical comparison because the authors have failed to report standard deviations (Table 2).

Table 2. Degree of Atherosclerosis in Habitual Coffee Drinkers and Habitual Tea Drinkers

	Coronary Atherosclerosis	Total Examined	Cerebral Atherosclerosis	Total Examined
Coffee drinking population (white)	58	155	33	150
Tea drinking population (Chinese)	37	50	12	40

From: YOUNG et al.: Tea and Atherosclerosis (Nature *216*, 1015 (1967).

In a total number of 150 examinations of cerebral arteries in Caucasians they found a mean degree of "33" in cerebral atherosclerosis and among 40 examinations of cerebral arteries in Chinese a mean degree of "12" in cerebral atherosclerosis.

Basically, we would agree to search for environmental factors in order to explain still existing differences in the rate and amount of atherosclerosis in the Caucasian and the Oriental race. But this particular study can not be called a search for factors, e. g. nutritional habits since the subjects were already deceased and were post mortem categorized into either white = "coffee drinking" or Chinese = "tea drinking".

It is quite an assumption to "lump" a Caucasian because of his white color into the "coffee drinking population", particularly after one has studied the coffee drinking habits of *white* people in our Evans County (Georgia) Study where we find actually more tea drinkers. However, in the absence of age- and sex-specific data and standard deviations of the grading of atherosclerosis of the coronary and cerebral arteries these numbers are useless and would lead us to become skeptical about the outcome of an animal experiment which this same group of investigators conducted. The purpose of their experiment was to explore the relationship between tea drinking and atherosclerosis (Table 3). Since tea contains a considerable amount of caffeine it is pertinent to discuss this experiment briefly.

Rabbits were maintained for 3 months on a diet consisting of rabbit chow augmented with 3% Wesson oil and 0.25% cholesterol. Data on serum lipids were available on 4 groups of animals but the frequency with which these determinations were made (or whether they represent a mean value) was not

Table 3. Relationship between Atherosclerosis and the Concentration of Serum Lipoproteins in Rabbits

Animal Group	(n)	Lipoproteins sf 0–12	12–20	20–100	Degree of Atherosclerosis
Control	(6)	50	16	0	0
Diet Alone	(5)	309	251	623	7.2
Diet and Tea	(4)	214	79	437	2.0
Diet early and Tea later	(10)	374	169	389	6.0

From: YOUNG et al.: Tea and Atherosclerosis (Nature *216*, 1015 (1967).

stated. The group of major interest was the one which was fed the diet plus tea. It consisted of 4 animals; the control group which received neither the high fat diet nor tea consisted of six animals and the group which received diet alone had 5 animals. Finally, the group which was on a diet 3 months before the tea was supplied in the drinking water had 10 animals. The table shows the results which led the investigators to the conclusion: "The data show that tea decreases concentration of lipids in the serum . . . they further suggest that if the atherosclerosis proceeds beyond a reversable stage, tea cannot reverse it. Probably tea must be supplied simultaneously with fat or immediately after a meal of fat. Theophylline and theobromine are far less effective, if at all, than tea in preventing the aorta from forming atheroma. Thus it seems that some active principle other than theophylline in the tea extract must be responsible for preventing the formation of atheroma". In view of the small number and variability of sample size, the absence of information on sex, age and weight of these animals and particularly in view of the preconceived hypothesis of this group of investigators, demonstrated in their study of coronary and cerebral atherosclerosis in man, we seriously doubt the validity of these conclusions.

We have participated in two experimental studies ourselves, both of which had a negative outcome as far as atherosclerosis of the aorta and the coronary arteries of rabbits or cholesterol metabolism were concerned (HEYDEN and RÜTTNER 1966 and HEYDEN et al. 1969). We have discussed our results with Dr. STAMLER in Chicago, who also found no hypercholesterolemia enhancing effect of coffee in chickens (personal communication, unpublished data).

Summary

For the past five years the medical literature dealing with coffee is replete with statements made by the two groups of investigators, LITTLE et al. and PAUL et al. quoted previously. From our discussion of the original articles and subsequently published papers by the same authors, it becomes clear that they do not support each other.

The statement by LITTLE and co-workers that the raised concentration of free fatty acids "may accelerate rate of synthesis and release of lipoproteins and raise serum lipid levels" is an assumption which they themselves questioned in these words: "It has yet to be shown, that other serum lipid fractions (besides free fatty acids) will become elevated with long-term drinking of coffee or that increased atherosclerosis results". PAUL and co-workers could not confirm the finding by LITTLE's group by stating: "We did not have evidence that the serum cholesterol level was significantly higher in the group with the highest coffee consumption". On the other hand LITTLE's group was unable to confirm the observation by PAUL's group that there was a significant correlation between the use of coffee and the later development of coronary disease in American patients. The Canadian coronary patients drank equal amounts of coffee as their controls and it was specifically mentioned that Toronto physicians have not prescribed modification of coffee ingestion in patients after myocardial infarction.

If therefore the two studies on which all future statements and speculations were based not only disagree but actually contradict each other we have good reasons to dismiss the issue at this time. This is an even more desirable decision as PAUL and co-workers themselves have retracted their 1963 statement when they admitted in 1968 that the importance of heavy use of coffee as a risk factor may be questioned. The fact that one of the most reliable epidemiological long-term studies, the Framingham Study, after 16 years of observation in over 5,000 adults could demonstrate "no substantial effect of heavy consumption of coffee or tea on morbidity and mortality" from coronary heart disease according to a 1967 report by KANNEL et al., favors our conclusion to dismiss the issue.

In addition we have attempted to demonstrate from several other conditions which cause an increment in free fatty acids that a subsequent rise in cholesterol levels is not observed. There is no reason to believe that the temporary elevation of free fatty acids after coffee ingestion necessarily leads to an increase in serum cholesterol concentrations. Our own observations in an animal species which is known to have a species specific susceptibility for hypercholesterolemia and atherogenesis convinced us that caffeine – regardless of the route of administration – over a three months period has no hypercholesterolemia enhancing effect at all.

In conclusion we feel the time has come to put an end to the allegations that coffee might exert a negative influence on the fat metabolism and its sequelae.

References

BELLET, S., A. KERSHBAUM, and E. M. FINCK, Metabolism 17, 702 (1968). — BELLET, S., A. KERSHBAUM, and L. ROMAN, Arch. Environ. Health 17, 803 (1968). — BELLET, S., L. ROMAN, R. O. DECASTRO, K. E. KIM, and A. KERSHBAUM, Metabolism 18, 288 (1969). — BLACKBURN, H., J. BROZEK, H. L. TAYLOR, and A. KEYS, Ann. J. N. Y. Acad. Sci. 90, 277 (1960). — BROWN, A., Brit. med. J. 5304, 567 (1962). — DOYLE, J., Postgrad. Med. 44, 188 (1968). — EPSTEIN, F., J. chron. Dis. 18, 735 (1965). — HEYDEN, S., Arch. Kreislaufforschg. 53, 1 (1967). — HEYDEN, S., W. DEMARIA, W. W. JOHNSTON, and W. M. O'FALLON, J. chron. Dis. 21, 677, (1969). — HEYDEN, S., Emotional Stress. In: G. SCHETTLER and G. S. BOYD, Atherosclerosis (Elsevier Amsterdam-New York 1969). — HEYDEN, S. and J. RÜTTNER, Pathol. Microbiol. 29, 291 (1966). — JACKSON, I. M. D., Metabolism 18, 13 (1969). — KANNEL, W. B., W. P. CASTELLI, and P. M. MCNAMARA, J. Occupat. Med. 9, 611 (1967). — KANNEL, W. B., The Framingham Heart Study, Habits and Coronary Heart Disease. P. H. S. Publication No. 1515 (Washington, D. C., 1966). — KERSHBAUM, A., S. BELLET, R. F. CAPLAN, and L. J. FEINBERG, Amer. J. Cardiol. 10, 204 (1962). — KERSHBAUM, A., R. KHORSANDIAN, R. F. CAPLAN, S. BELLET, and L. J. FEINBERG, Circulation 28, 52 (1963). — LEVI, L., Das Experiment am Menschen in der Psychosomatik. Verh. Dtsch. Ges. Inn. Med. 73, 582 (München 1967). — LITTLE, J. A., H. M. SHANOFF, A. CSIMA, S. E. REDMOND, and R. YANO, Lancet I, 933 (1965). — LITTLE, J. A., H. M. SHANOFF, A. CSIMA, and

R. YANO, Lancet I, 732 (1966). — NAISMITH, D. J., P. A. AKINYANJU, and J. YUDKIN, Proceedings of the Nutr. Soc. 28, 12A (Abstracts of Communications) (1969); J. Nutrition 97, 375 (1969). — PAUL, O., Postgrad. Med. 44, 196 (1968). — PAUL, O., M. H. LEPPER, W. H. PHELAN, G. W. DUPERTUIS, A. MACMILLAN, H. MC KEAN, and H. PARK, Circulation 28, 20 (1963). — PAUL, O., A. MACMILLAN, H. MCKEAN, and H. PARK, Lancet II, 1049 (1968). — RAAB, W. and H. J. KREYWANEK, Cardiac Sympathetic Tone and Stress Response Related to Personality Patterns and Exercise Habits. WILHELM RAAB (Ed), Prevention of Ischemic Heart Disease: Principles and Practice (Springfield, Illinois 1966). — RODAHL, K., H. I. MILLER, and B. ISSEKUTZ Jr., J. Appl. Physiol. 19, 489 (1964). — VOLKHEIMER, G., F. H. SCHULZ, E. HOFER und J. SCHICHT, Nutr. Dieta 11, 13 (1969). — WALKER, W. J. and G. GREGORATUS, Amer. J. Card. 19, 339 (1969). — ZELLER, W. und H. P. T. AMMON, Z. Gastroenterologie 5, 84 (1967). — YOUNG, W., R. L. HOTOWEC, and A. G. ROMERO, Nature 216, 1015 (1967).

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CAFFEINE EFFECTS ON CHOLESTEROL AND DEVELOPMENT OF AORTIC AND CORONARY ATHEROSCLEROSIS IN RABBITS

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INTRODUCTION

RECENT reports have dealt with the possible lipid elevating effect of coffee or caffeine. One group's [1] conclusions were as follows: "The data suggest that coffee contains a substance which elevates serum-lipids in susceptible persons and that such persons may be liable to coronary heart disease". These authors, however, noted that "it has yet to be shown that other serum lipid fractions (besides plasma non-esterified fatty acids) will become elevated with long-term drinking of coffee or that increased atherosclerosis results". Another study [2] revealed a rise of free fatty acids in human subjects after drinking two cups of coffee. Since de-caffeinated coffee showed no effect it was suggested [3] that caffeine is the lipolytic ingredient of coffee and further—"These findings are of interest in view of the relationship of free fatty acid elevation to that of other lipid fractions, notably triglycerides and cholesterol". In contrast to these assumptions other authors [4, 5] observed in animal experiments that caffeine did not have a cholesterol increasing effect in cholesterol-fed rabbits and that these rabbits did not show more aortic atherogenesis than the control animals. Confirmation of these results was reported by one of us (S.H.)[6].

The aim of this study was to observe any changes in blood cholesterol and atherogenesis associated with administration of caffeine in rabbits who were maintained on an atherogenic diet.

METHOD

Three groups of male, white New Zealand rabbits, weighing 1.5–2 kg were used. The animals in group A were fed standard rabbit diet to which an atherogenic diet (see later) and 50 mg of instant coffee per day were added. In group B the animals were placed on the same standard and atherogenic diets but without coffee. Instead the animals received daily subcutaneous injections of 0.22 cm^3 caffeine benzoate (25 mg caffeine). This dosage was chosen purposely to be small compared to the toxic caffeine doses for rabbits (about 325 mg per kg body weight for oral feeding and 150 mg per kg body weight for subcutaneous injection). The animals in group C

The atherogenic diet was prepared weekly for the entire group by dissolving powdered cholesterol in hot corn oil followed by thorough mixture of oatmeal in this solution. This diet was stored in the refrigerator and each morning every animal was given a measured amount containing 1 g of cholesterol dissolved in 1 cm^3 of corn oil and incorporated into 2 g of oatmeal.

The animals were all housed in individual cages in the same animal room and fed and handled by the same personnel. The animals were bled four times for cholesterol determination, after establishing the base line in two determinations prior to the experiment.

At the termination of the experiment all animals were sacrificed and the hearts and aortas removed. The grading of aortic atherosclerosis was done by three observers independently without prior knowledge to which group the aortas belong. Absence of any changes was graded 0; spotty lesions were graded 1+, confluent lesions covering parts of the aorta 2+, confluent lesions covering more than half of the aorta 3+, and confluent lesions covering almost the entire aorta 4+. Only a few cases were graded 5+ showing lesions which covered the entire aorta. No ulcerating lesion was seen. In cases of disagreement between the three observers, the aortas were rearranged and reviewed until two observers agreed. Each heart was sectioned transversely at 2 mm intervals from base to apex and fixed in 10 per cent formalin. The first three transverse sections from each heart were submitted for paraffin blocks. A hematoxylin and eosin section was cut from each block. The degree of coronary atherosclerosis was evaluated microscopically in three ways. In the three hematoxylin and eosin sections from each heart all arterial sections were studied and counted. An estimate of the extent of arterial disease was obtained from the ratio of diseased arterial sections to total arterial sections counted. The results of these counts are shown in Table 1.

TABLE 1. EXTENT OF ATHEROSCLEROSIS IN CORONARY ARTERY SEGMENTS

Animal Number	Number atherosclerotic coronary arteries / Total coronary arterial count	Percentage atherosclerotic involvement
B1	20/37	54
B2	20/34	59
B3	19/36	53
B4	54/59	90
B5	37/45	82
B6	32/39	82
B7	15/24	63
B8	20/30	67
B9	20/30	67
C1	40/48	80
C2	38/47	81
C3	27/32	84
C4	52/63	83
C5	27/36	75
C6	21/34	62
C7	23/31	74
C8	15/26	60
C9	29/39	72
C10	31/42	74
C11	49/63	78
C12	7/12	60

TABLE 2. SEVERITY OF ATHEROSCLEROTIC OCCLUSION OF CORONARY ARTERIES

Animal number	Percentage arteries with 1+ occlusion	Percentage arteries with 2+ occlusion	Percentage arteries with 3+ occlusion
B1	4	4	22
B2	5	0	26
B3	11	0	11
B4	11	22	35
B5	9	14	0
B6	12	6	22
B7	35	3	13
B8	17	9	34
B9	5	5	47
C1	19	6	28
C2	12	9	22
C3	22	0	4
C4	14	12	33
C5	24	8	24
C6	3	3	37
C7	5	10	5
C8	3	3	7
C9	13	3	13
C10	7	4	54
C11	2	4	25
C12	10	5	14

TABLE 3. SEVERITY OF ATHEROSCLEROTIC OCCLUSION OF EPICARDIAL BRANCHES OF LEFT AND RIGHT CORONARY ARTERIES

Animal number	Grade of arterial involvement with atherosclerosis			
	0	1+	2+	3+
B1				X
B2	X			
B3	X			
B4	X			
B5	X			
B6				X
B7		X		
B8			X	
B9	X			
C1		X		
C2	X			
C3	X			
C4			X	
C5			X	
C6	X			
C7		X		
C8	X			
C9			X	
C10				X
C11			X	
C12		X		

TABLE 4. MEAN SERUM CHOLESTEROL LEVEL INCREASES

		Group		
		A	B	C
Base line	Mean	118.1	117.7	147.3
	S.D.	38.1	32.1	51.9
	Sample size	11	10	13
3-week increase	Mean	490	978	935
	S.D.	403	691	492
	Sample size	*9	10	13
6-week increase	Mean	799	1019	1243
	S.D.	734	454	489
	Sample size	10	10	13
9-week increase	Mean	701	1211	1601
	S.D.	485	442	666
	Sample size	9	9	12
12-week increase	Mean	756	1350	1448
	S.D.	364	436	536
	Sample size	9	9	12

*Differences in sample size due to deficient specimens and/or mortality.

As a second parameter for evaluating coronary arterial disease the arterial sections were graded for degree of arterial occlusion on a 0–3+ scale. A grade of 0 was assigned to any arterial section showing less than 5 per cent occlusion, 1+ to any vessel showing 5–30 per cent occlusion, 2+ to any vessel showing 30–60 per cent occlusion, and 3+ to any vessel showing 60–100 per cent occlusion. The results of this grading are shown in Table 2. As a third parameter for evaluating coronary artery disease the major epicardial branches of the left and right coronary arteries were studied for degree of atherosclerotic occlusion using the same grading system. These results are shown in Table 3.

RESULTS

Within a few weeks the cholesterol levels rose above base line in the rabbits in all three groups as anticipated. Figure 1 illustrates the regression lines obtained when this increase was regressed against time (see Appendix for details) in each of the three groups. In addition, the points plotted in Fig. 1, represent the mean increase at each time and provide an indication of the goodness-of-fit of the lines. Table 4 presents these mean increases along with standard deviations and sample sizes.

Analysis of the regression lines and other statistical procedures (see Appendix) indicated that the only significant differences existing between groups were differences between groups A and C at the later times of observations. Since, for reasons explained below, group A was removed from further consideration, these differences became irrelevant and the basic result of the statistical analyses is that no significant differences could be found between groups B and C.

The food was readily eaten by the animals in groups B and C, group A animals initially refused to eat this mixture but subsequently all animals ate most of this diet.

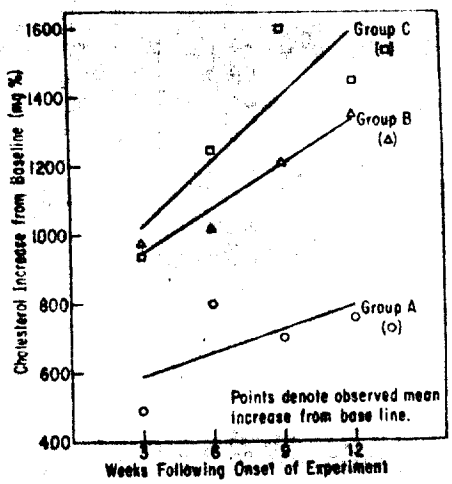

FIG. 1.

However, the fact that the rabbits in group A did not eat the entire amount makes interpretation of this difference impossible. It could be due either to the added coffee or to the decreased cholesterol intake. Therefore, the remainder of this report will be confined to a discussion of the results obtained in groups B and C.

The animals of experimental group B did eat the same amount of cholesterol-treated oatmeal as the animals of control group C. The degree of hypercholesterolemia developing over the 12-week period was higher in group C when compared to group B, but the difference is not significant.

There was no significant difference in the degree of atherosclerosis of the aorta in the caffeine-treated group and the control group, with only a few more control animals showing more extensive atherosclerosis of the aorta.

Microscopic examination of the coronary arteries showed atherosclerotic changes which are characteristic for rabbits fed a diet rich in cholesterol. There was a generalized involvement of the coronary arterial tree with lipid deposits lying both free in the tissues and in macrophages. In some vessels, the lesions were limited to the intima and were characterized by aggregations of lipid-filled macrophages forming a plaque. In other vessels, the lesions were much more extensive and involved the entire thickness of the arterial wall. Heavy concentrations of lipids were also observed in the sub-endocardial tissues and myocardium. Extent and severity of lipid involvement increased as vessel size decreased.

From Table 1 it can be seen that the percentage of artery sections in each animal heart showing atherosclerosis varied from animal to animal both within group B rabbits and the group C rabbits. Thus, atherosclerosis of coronary arteries averaged 71 per cent in the caffeine-treated rabbits and 76 per cent in the control rabbits. The differences between the animal groups are not statistically significant. Likewise, Table 2 reveals marked fluctuation between animals in each group in the severity of arterial occlusion. Again, the differences between the caffeine-treated rabbits and the control rabbits are not statistically significant.

Table 3 shows that three out of nine caffeine-treated animals (33 per cent) had

2+ to 3+ occlusive disease of the epicardial vessels, while 5 out of 12 control rabbits (41 per cent) had 2+ to 3+ occlusive disease.

DISCUSSION

We interpret these results as adding to our previously taken position [6] that the administration of caffeine—if it has any effect in rabbits—has no hypercholesterolemia enhancing effect. The observation of the higher individual cholesterol levels as well as the higher mean cholesterol level in the control group and not in the caffeine-treated group has to be weighed in the light of the following:

(a) The rabbit is known to have a species specific susceptibility for hypercholesterolemia and its sequelae. If caffeine would indeed have a hypercholesterolemia enhancing effect as proposed in the recent statements mentioned in the introduction, we would have expected a higher mean cholesterol level in the caffeine treated group than in the control group.

(b) The dosage of the daily caffeine injections for rabbits can be compared with a very high coffee consumption in man.

At the onset the rabbits weighed 1.5–2 kg, therefore the daily caffeine dosage on a body weight basis was 12.5–16.7 mg. The average cup of coffee probably contains about 100 mg of caffeine and in a 70 kg man this is about 1.4 mg of caffeine per kg of body weight. Assuming no differences in species sensitivity, this translates the rabbit dose to a daily human coffee intake equivalent of 9–12 cups. Although it could be argued that such an intake is "excessive" it is certainly not out of the realm of reality. Furthermore our concern, if any, would probably be with individuals who do consume coffee at these levels. Caffeine levels which might be considered high in relation to average human consumption (2.87 cups per day) thus had no exacerbating effects on serum cholesterol or atherosclerosis.

The results reported in our caffeine–benzoate injection group are similar to those reported by KEDRA *et al.* [7] with administration of caffeine–benzoate by mouth. They summarized their second 3-months' experiment which involved 18 rabbits receiving 1 g. of cholesterol plus 80 mg of caffeine–benzoate in water per day and 17 control animals receiving the same amount of cholesterol without caffeine as follows: "This study showed that atherosclerosis is more extensive in cholesterol-fed rabbits (mean index 2.32) than in those simultaneously treated with cholesterol and caffeine (mean index 1.61). However, the difference is not statistically significant . . . It is a fact that the rabbits of this group (cholesterol + caffeine) exhibited a lower level of total lipids, cholesterol and lipoproteins than those of the control group. Again, the differences are statistically insignificant". The coronary arteries have not been examined in previous animal experiments. There was no increase either in the extent or the severity of atherosclerosis of the coronary arteries in the caffeine-treated animals in our experience.

Interpretation of these combined results lead us to state that in spite of administration of 25 mg of caffeine for a considerable length of time and in spite of the well known high susceptibility of rabbits for the development of hypercholesterolemia and atherosclerosis, the proposed cholesterol-elevating effect of coffee was not observed.

These results of animal experimentation cannot be directly applied to the process of atherogenesis and the development of both hypercholesterolemia and clinical ischemic heart disease in man. We would also hesitate to accept without confirmation

a single epidemiological observation showing a positive relation between coffee intake and coronary disease as reported by PAUL *et al.* [8]. They noted from a longitudinal study of coronary heart disease, that a "significant correlation between the use of coffee and the later development of coronary disease is seen ($p<0.025$)". It soon became a widely accepted statement regarding potential dangers of excessive coffee drinking. This finding has not been validated in three other epidemiological studies: BROWN (Cheshire, England) [9] investigated the records of 536 former patients presumed to have died as a result of coronary heart disease and compared the coffee consumption of these patients with a group of 642 controls believed to have died from variable causes. It appeared from this retrospective investigation that the coronary group consumed somewhat less coffee than the controls. The Framingham Heart Study, reported by KANNEL [10] has failed to show a significant association in either sex between coronary heart disease and coffee consumption. WALKER and GREGORATOS [11] studied 100 male military personnel aged 40 or less at the time of myocardial infarction and compared them with an aged-matched military control group. Fifty-nine per cent of the infarct group consumed more than 4 cups of coffee a day as compared to 51 per cent of the control group, not a significant difference.

The data in the paper by PAUL *et al.* [8] are based on information of coffee drinking habits gathered from 1108 non-coronary men and 54 "coronary cases". The statement relating the development of coronary disease to excessive coffee consumption (5 or more cups per day) is based on 19 per cent which amounts to 10 men out of 54 patients. In the control group, 78 men of 1108 non-coronary males exceeded the average use of coffee to this same extent. Unfortunately, the 54 "coronary cases" are not categorized as patients with either angina pectoris, myocardial infarction or patients who died from coronary disease. Even if taken at face value, the effect on the coronary arteries cannot necessarily be attributed to the coffee intake because the competing effect of other factors, e.g. nicotine inhalation has not been taken into account. We have observed that those individuals who are excessive coffee drinkers and smokers, in general, tend to smoke cigarettes excessively (personal observation, unpublished data). If this observation is true also in the Chicago study, the authors cannot rule out the known detrimental influence of nicotine inhalation on the development of ischemic heart disease. Further, it would seem necessary to inquire in more detail whether the excessive coffee consumption has been a lifelong habit or whether it was only a temporary custom.

It is interesting to note that ZELLER and AMMON [12] were unable to change the cholesterol level or the level of esterified fatty acids in the serum of 52 patients with liver disease, as well as 41 healthy control persons after injections of caffeine or oral ingestion of coffee. These results were obtained in six laboratory determinations on each subject over a period of 3 hr after intravenous application of 250 mg of caffeine or consumption of 225 mg of caffeine. However, as expected, they saw a marked elevation of free fatty acids in healthy persons with a peak serum level 2 hr after caffeine administration and a peak serum level 1–6 hr after caffeine administration in patients with liver disease. It is known that caffeine stimulates an increased catecholamine secretion into the adrenal venous blood and exerts a very significant effect on adrenaline excretion and to a lesser extent on noradrenaline excretion into the urine[13]. To assume that this would have an effect on the cholesterol metabolism

because of the obvious catecholamine induced effect on the free fatty acids, seems unjustified at this time.

SUMMARY

Three epidemiological studies have not revealed any relationship between coffee consumption and the development of ischemic heart disease. One longitudinal study of ischemic heart disease implied that this relationship did exist.

Others have shown in several long-term animal experiments with caffeine administration that no hypercholesterolemia-enhancing effect of caffeine exists. Short-term clinical trials in healthy individuals and patients with liver disease who received caffeine both intravenously and orally produced no changes in the cholesterol levels. Previously reported animal experiments have not resulted in severer degrees of atherosclerosis or more extensive involvement of the aortas in caffeine-treated animals compared to control animals. This report clearly demonstrates no increase in coronary atherosclerosis in caffeine-treated animals.

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REFERENCES

1. LITTLE, J. A., SHANOFF, H. M., CSIMA, A. and YANO, R.: Coffee and Serum-lipids in coronary heart disease. *Lancet* **1**, 732 1966.
2. BELLET, S., ASPE, J., KERSHBAUM, A. and ZANUTTINI, D.: Effect of caffeine on free fatty acids. *Circulation* **29-30**, 45, 1964.
3. BELLET, S., KERSHBAUM, A. and FINCK, E. M.: Effect of caffeine on free fatty acids: Mechanisms of effect. *Circulation* **33-34**, 53, 1966.
4. CZOCHRA-LYSANOWICZ, A., GORSKI, M. and KEDRA. M.: The effect of nicotine and caffeine on the development of arteriosclerosis in rabbits. *Annls Univ. Mana. Curie-Sklodowska* **14**, 181, 1959.
5. KEDRA, M.: Influence of caffeine and nicotine on the development of arteriosclerosis in rabbits. *Pol. Archs. Med.* **30**, 1004, 1960.
6. HEYDEN, S. and RÜTTNER, J.: Die Beeinflussung des Cholesterinspiegels durch Coffein im Tierversuch (The influence of caffeine on the cholesterol levels in animal experiments). *Path Microbiol.* **29**, 291, 1966.
7. KEDRA, M., CHIBOWSKI, D. and POLESZAK, J.: Effect of caffeine on the development of atherosclerosis in cholesterol-fed rabbits. *Pol. Med. J.* **6**, 352, 1967.
8. PAUL, O., LEPPER, M. H., PHELAN, W. H., DUPERTUIS, G. W., MACMILLAN, A., MCKEAN, H. and PARK, H.: A longitudinal study of coronary heart disease. *Circulation* **28**, 20, 1963.
9. BROWN, A.: Coronary thrombosis; an environmental study. *Br. med. J.* **5304**, 567, 1962.
10. KANNEL, W. B.: The Framingham Heart Study. *Habits and Coronary Heart Disease.* P.H.S. Publication No. 1515, U.S. Department of Health, Education and Welfare, N.H.I.—U.S. Govt. Print. Office, 1966.
11. WALKER, W. J. and GREGORATOS, G.: Myocardial infarction in young men. *Am. J. Cardiol.* **19**, 339, 1967.
12. ZELLER, W. and AMMON, H.P.T.: Der Einfluss von Koffein auf den Fettstoffwechsel bei Lebererkrankungen (Influence of caffeine on fat metabolism). *Z. Gastroenterologie* **5**, 84, 1967.
13. LEVI, L.: The effect of coffee on the function of the sympathoadrenomedullary system in man. *Acta med. scand.* **181**, 431, 1967.

APPENDIX

The regression lines plotted in Fig. 1 are:

Group A	$519.1+22.8t$
Group B	$813.1+43.4t$
Group C	$827.0+63.8t$

Each line was tested for the "lack of fit" of the linear model and no significant indication of a lack of fit was found. However, the lines accounted for only 22, 8 and 13.5 per cent of the variation in

groups A, B and C respectively. Neither the slopes nor the intercepts of the lines differ significantly from each other when appropriate multiple comparison tests are made. In addition, the only slope which is definitely significantly different from 0 is the group C slope although the group B slope has a p-value between 0.1 and 0.05.

These facts would indicate that at best the regression lines would be useful only in indicating a very vague linear trend over the 9-week period of observation which cannot be shown to be different for the three groups.

Since no group differences were apparent from this analysis further analyses were carried out in an attempt to discover any differences which might exist. Barlett's test for homogeneity of the three group variances for the baseline observations as well as for the four increases above baseline failed to show any significant differences between variances. These tests were followed by five separate (but not independent) analyses of variance testing the equality of the three group means for the baseline measurements and for the 3, 6, 9, and 12-week increases. At both 9 and 12 weeks the three means were significantly different ($p<0.01$) but this p-value must be interpreted conservatively because of the effect of doing several dependent tests on the true significance levels. For these two occasions, the three means were compared two at a time and in each instance only the groups A and C means were significantly different ($p<0.01$ at 9 weeks and $p<0.05$ at 12 weeks).

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Coffee Consumption from a Medical Viewpoint

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During the past two years, the non-professional press--stimulated by various publications of the professional medical literature--published concern and warnings against coffee consumption, which require urgent clarification. A brief comment is intended here on the basis of the most recent scientific investigations concerning concepts which contend that coffee, and in particular caffeine, can be "harmful" for hypertonics and gout patients, diabetics and heart infarct patients.

Survey of epidemiologic and experimental research results.

1. Hypertonia.

At Farmingham (1) coffee consumption was correlated with blood pressure in 5000 adults. "Evidence that the temporary slight increase of the blood pressure which can be induced by caffeine has a cumulative effect on the elevation of the blood pressure, i.e. that it makes the patient susceptible to definite, manifest hypertonia, is lacking. The examination of the correlation coefficients between habitual coffee consumption and systolic blood pressure (0,04) did not even reveal any tendency toward an association between coffee consumption and the development of hypertonia".

As known, a prospective incidence-study is at issue at Farmingham, while we report on prevalence, on the basis of the Evans County-study (2), of various findings. Men with high coffee consumption (5 cups per day and more) had an average blood pressure of 137/85 mm Hg.

men who consumed little or no coffee had a blood pressure around 147/88 mm Hg; identical correlations were found in Negroes. The above finding of an insignificant, but consistently higher blood pressure in all four racial and sex groups with little or no coffee consumption --as compared with strong coffee consumption--can be explained by the observed larger number of overweight persons. Those who consume large amounts of coffee are also frequently stronger cigaret smokers as well, with lower average weight than the non-smokers.

Pharmecologists likewise discussed the caffeine effect on blood pressure: " Blood pressure is hardly affected by caffeine; occasionally,a sleight drop occurs, which can be followed by a phase of increased pressure"(3).

A recently published Manual of Pharmacology (4) states: "Caffeine and theophylline in large doses stimulate the vessel- and respiratory centers. Neverthless, the blood pressure does not rise because the vessels of the skin, kidney and of the heart are dilated as a result of a peripheral attack. . . Caffeine quantities of 0,05 to 0,2 g per os, contained in 1-2 cups of coffee or tea inhibit falling asleep and uninterrupted sleep respectively, after retiring. Paradoxically, caffeine can facilitate falling asleep in hypertonics and in the aged."

Or, citing from American literature(5): "The majority of scientists is now convinced that the prohibition of coffee consumption is worse for the hypertonic who likes coffee than stimulation by xanthine-containing beverages. Most hypertonia patients experience no blood pressure increase or nervousness. The xanthines can furthermore inhibit headache which is so often observed in cases of hypertonia, or can mitigate the same."

2. Hyperuremia and gout

It was often erroneously assumed that the metabolic end-products of caffeine result in increased uric acid concentration in the serum. At County Evans 2530 persons were questioned concerning their coffee intake habits; they were divided into groups of strong coffee consumers (an average of 5 cups or more per day), and persons consuming little or no coffee (2). The uric acid level of the men was 5,5 mg% in case of strong coffee consumption, and 5,6 mg% in case of minor coffee consumption; in women, the rates were 4,4 mg% and 4,8 mg% respectively; findings in Negroes were identical. Accordingly, no difference can be found regarding the uric acid level of a population group when subdivided into two coffee consumption groups. On the other hand, numerous indications exist concerning causal correlations between hyperuremia and overweight, hyperuremia and excessive alcohol consumption; indications also exist concerning drug-induced hyperuremia; in other words: hyperuremiae which are in most cases largely reversible when the causative factors are eliminated.

Pharmacologists stated as well (5): " The xanthines are only partially demethylated and oxidized in the body. They are principally excreted as methyluric acid or as methylxanthine. It was determined already in 1957 that in man theophylline is initially converted into 1.3-dimethyl-uric acid: theobromine is excreted as 3-methyl- as well as 7-methylxanthine; caffeine is excreted as 1-methyl-uric acid and 1-methylxanthine, in relatively equal amounts. Approximately 10 % of this alkaloid is excreted unchanged. Since none of the xanthines is completely demethylated, the excretion of the uric acid as such does not increase. This important fact indicates that the xanthines are not contraindicated for gout patients."

3. Diabetes mellitus.

The advertising of decaffeinated coffee--based on the assumptions of an American scientist--unfortunately contributes to

the doubts of diabetics concerning the extent of the permitted coffee consumption (6). The advertisements state, among others, that many diabetics believe coffee to be innocuous for them "while recent scientific findings made such a view neverthless highly questioable". It is stated that " the coffee content in particular has a negative effect on the metabolism of diabetics". This adverstisement is intended, among others, as a warning for millions of diabetics that " caffeine is an additional load for Beta-cells in the diabetic". Furthermore, caffeine allegedly can "intensify" diabetes symptoms. 2 cups of coffee with standard caffeine content can cause a definite increase of the blood sugar level and of the acid values in the diabetic".

After finding in the Evans County Study (2) statistically non-significant differences between strong coffee consumers and persons who consumed no coffee or only minor quantities of the same (men with strong coffee consumption: average of 100 mg% fasting blood sugar; men with minor or no coffee consumption: 93 mg%; women with high coffee consumption: 98 mg% and women with minor or no coffee consumption: 94 mg%), we determined additionally that strong coffee consumers and those who consumed no coffee had contracted diabetes mellitus with the same frequency. We therefore agree with the opinion of MEHNERT (personal information, 1973): "The question whether caffeine is harmful for diabetics can be unequivocally answered in the negative. While it is true that caffeine promotes insulin secretion from the pancreas, no harmful effect--in the sense of insulin production depletion -- was ever found. Fundamentally, the effect of caffeine is rather comparable to the effect of drugs (such as for example: sulfonamid-preparations) which are prescribed for diabetics to induce improved metabolism. This, however, cannot mean that caffeine is used for diabetes therapy (for which purpose the effect is too weak). But no reason whatsoever exists for prohibiting coffee containing caffeine. Our hands as physicians are regrettably tied in many respects in dietetic diabetes therapy: we have to prohibit or restrict

some items which are favored by the patients; the list of prohibited (food and beverage) items should therefore not be extended needlessly to those enjoyed by patients against which no objections can be raised".

4. Myocardial infarct.

A report (7) was published in 1972 by the Boston Collaborative Drug Surveillance Program on the subject of coffee consumption and acute myocardial infarct. According to said Report, women who drink coffee are assumed to be 4 times more susceptible to heart infarct than women who drink no coffee, and chain smokers are allegedly less susceptible to infarct risk than coffee consumers! In 1973 the same research group published additional results: it was found, after interviewing 440 heart infarct patients and more than 12 000 hospitalized patients as control group, that--as compared with those who do not drink coffee--"the daily intake of 1 to 5 cups of coffee involves a 60 % higher infarct risk, and a risk is 120 % when more than 6 cups of coffee are consumed per day". Surprisingly, tea consumption and infarct are not correlated (as known, 1 cup of tea, as does 1 cup of strong coffee, contains 120 to 150 mg caffeine!). Said study is retrospective as well, and limited to hispitalized patients. Since those patients who died as a result of heart infarct prior to hospital admission cannot be included, the question arises how far the infarct group is representative, since the disease in question has a 30% mortality rate prior to hospitalization. Furthermore, patients who died shortly after hospitalization were not interviewed either. It should likewise be questioned how far the control group is representative. As the authors of the Farmingham study remark in their editorial (9), control patients from the various medical and surgical departments of numerous clinics are certainly not comparable to the general population.

Percentually, an unusually high number of persons could be among them who refrained from coffee drinking for some reason, either voluntarily or at doctor's orders. The Boston colleagues(8) state very cautiously that a causative explanation of the coffee consumption-myocardial infarct correlation is not justified on the basis of their results. The results of the retrospective investigations are unequivocally opposite to those of the prospective Chicago studies (10) and to the Farmingham studies (9). In a second publication (11) PAUL had to admit that coffee consumption alone is not responsible for the higher infarct rate among coffee drinkers; instead, cigaret smoking is held responsible. In the Gothenburg study--likewise prospective, for a period of 8 years--no correlation was verified between coffee drinking habits and heart infarct. (WILHELMSEN and TIBBLIN, in print, 1973). Another retrospective study (2) compared the infarct group with the healthy population which served as control group. No difference whatsoever was found between heart infarct patients and healthy individuals in correlation with coffee consumption.

Final conclusion: From a medical viewpoint, no reason exists for restricting the coffee consumption of patients either in case of hypertonia and gout patients, or diabetics nor during primary and secondary heart infarct prophylaxis.

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1. DAWBER T. R., KANNEL W. B., KAGAN A., DONABEDIAN R. K., MCNAMARA P. M. und PEARSON G.: Environmental factors in hypertension. In: Epidemiology of hypertension, S. 225–288. Grune & Stratton, New York 1967.
2. HEYDEN S., BARTEL A., CASSEL J. C., HAMES C. G., TYROLER H. A. und MIER R.: Kaffeekonsum, Gefässerkrankungen und Risikofaktoren in der Evans-County/Georgia-Studie. Z. Ernährungswiss., Suppl. 14, S. 1–10 (1972).
3. HAUSCHILD F. und GÖRISCH V.: Einführung in die Pharmakologie und Arzneiverordnungslehre, S. 317–319. Thieme, Leipzig 1967.
4. KUSCHINSKY G. und LÜLLMANN H.: Kurzes Lehrbuch der Pharmakologie, 5. Aufl. Georg Thieme Verlag, Stuttgart 1972.
5. GOODMAN L. S. und GILMAN A.: The pharmacological basis of therapeutics, 4th Ed. MacMillan Company, New York 1970.
6. HAG AG, Bremen: Kaffee für Diabetiker? In: Bircher-Benner, Handbuch Nr. 14 (1973).
7. Boston Collaborative Drug Surveillance Program: Coffee drinking and acute myocardial infarction. Lancet *1972*/II, 1278–1281.
8. JICK H., MIETTINEN O. S., NEFF R. K., SHAPIRO S., HEINONEN O. P. und SLONE D.: Coffee and myocardial infarction. New Engl. J. Med. *289*, 63–67 (1973).
9. KANNEL W. B. und DAWBER T. R.: Coffee and coronary disease. Editorial. New Engl. J. Med. *289*, 100–101 (1973).
10. PAUL O., MACMILLAN A., MCKEAN H. und PARK H.: Sucrose intake and coronary heart disease. Lancet *1968*/II, 1049.
11. PAUL O.: Stimulants and coronaries. Postgrad. Med. *44*, 196 (1968).

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Abteilung Präventivmedizin, Sanitätsdepartement St. Gallen

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Kaffeekonsum aus ärztlicher Sicht

S. Heyden und M. Escher

In den vergangenen zwei Jahren sind in der Laienpresse – stimuliert durch verschiedene Veröffentlichungen in der medizinischen Fachliteratur – Bedenken und Anfragen gegen den Kaffeekonsum in die Öffentlichkeit getragen worden, die dringend einer Klärung bedürfen. Die Vorstellungen, dass Kaffee und insbesondere Koffein für Hypertoniker und Gichtkranke, Diabetiker und Herzinfarktpatienten «schädlich» seien, sollen auf Grund der neuesten wissenschaftlichen Untersuchungen kurz kommentiert werden.

Übersicht über epidemiologische und experimentelle Forschungsergebnisse

1. Hypertonie

In Framingham [1] wurde bei 5000 Erwachsenen der Kaffeekonsum mit dem Blutdruck korreliert: «Ein Hinweis, dass die vorübergehende leichte Erhöhung des Blutdrucks, wie sie durch Koffein hervorgerufen werden kann, einen kumulativen Effekt auf die Höhe des Blutdrucks habe, d. h. für eine fixierte, manifeste Hypertonie prädisponiere, fehlt. Die Prüfung des Korrelationskoeffizienten zwischen dem gewohnheitsmässigen Kaffeeverbrauch und dem systolischen Blutdruck (0,04) zeigte nicht einmal eine Tendenz für eine Assoziation zwischen Kaffeekonsum und der Entwicklung einer Hypertonie.»

Bekanntlich handelt es sich in Framingham um eine prospektive *Inzidenz*-Studie, während wir aus der Evans County-Studie [2] über die *Prävalenz* verschiedener Befunde berichteten. Männer mit hohem Kaffeekonsum (5 Tassen pro Tag und mehr) hatten einen durchschnittlichen Blutdruck von 137/85 mm Hg, Männer mit geringem oder keinem Kaffeekonsum von 143/88 mm Hg; Frauen 139/85 mm Hg bzw. 147/88 mm Hg; bei Negern fanden sich gleiche Verhältnisse. Dieser Befund eines zwar insignifikant, aber konsistent höheren Blutdrucks in allen vier Rassen- und Geschlechtsgruppen bei Personen *mit geringem oder keinem Kaffeeverbrauch* – im Vergleich zu den starken Kaffeekonsumenten – kann durch die beobachtete grössere Anzahl an übergewichtigen Personen erklärt werden. Die starken Kaffeetrinker sind häufig gleichzeitig die stärkeren Zigarettenraucher mit niedrigeren Durchschnittsgewichten als die Nichtraucher.

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Pharmakologen haben sich ebenfalls mit der Koffeinwirkung auf den Blutdruck auseinandergesetzt: «Der Blutdruck wird durch Koffein kaum beeinflusst, mitunter findet sich leichte Senkung, an die sich eine Phase der Druckerhöhung anschliessen kann» [3].

In einem kürzlich erschienenen Lehrbuch der Pharmakologie [4] heisst es: «Koffein und Theophyllin erregen in grossen Dosen Gefäss- und Atemzentren. Trotzdem steigt der Blutdruck nicht an, weil auf Grund eines peripheren Angriffs die Gefässe von Haut, Niere und Herz erweitert werden ... Koffeinmengen von 0,05 bis 0,2 g per os, die in 1–2 Tassen Kaffee oder Tee enthalten sind, verhindern nach dem Schlafengehen das Ein- bzw. Durchschlafen. Bei alten Menschen und manchmal bei Hypertonikern kann Koffein paradoxerweise das Einschlafen erleichtern».

Oder aus der amerikanischen Literatur [5]: «Die Mehrzahl der Wissenschaftler ist jetzt der Überzeugung, dass das Kaffeeverbot für einen Hypertoniker, der Kaffee gerne trinkt, schlimmer ist als die Stimulierung durch xanthinhaltige Getränke. Die meisten Patienten mit Hypertonie erfahren weder einen Anstieg ihres Blutdrucks noch Nervosität. Die Xanthine können darüber hinaus oft noch den Kopfschmerz, der so häufig bei der Hypertonie beobachtet wird, verhindern oder verbessern».

2. Hyperurikämie und Gicht

Fälschlicherweise wurde häufig angenommen, dass die metabolischen Endprodukte des Koffeins zu einer Erhöhung der Harnsäurekonzentration im Serum führen. In Evans County wurden 2530 Personen nach ihren Kaffeetrinkgewohnheiten befragt und in starke Kaffeekonsumenten (durchschnittlich 5 Tassen und mehr pro Tag) und Personen mit geringem oder keinem Kaffeekonsum eingeteilt [2]. Die Harnsäurespiegel der Männer betrugen 5,5 mg% bei starkem und 5,6 mg% bei geringem Kaffeekonsum; bei Frauen 4,4 mg% bzw. 4,8 mg%; bei Negern identische Befunde. Somit ist kein Unterschied in den Harnsäurespiegeln einer Bevölkerung bei Unterteilung in zwei Kaffeekonsumgruppen festzustellen. Dagegen existieren zahlreiche Hinweise auf die kausalen Zusammenhänge zwischen Hyperurikämie und Übergewicht, Hyperurikämie und exzessivem Alkoholkonsum sowie auf medikamente-induzierte Hyperurikämie – mit anderen Worten Hyperurikämien, die bei Ausschaltung der verursachenden Faktoren meistens weitgehend reversibel sind.

Pharmakologen bezogen ebenfalls Stellung [5]: «Im Körper werden die Xanthine nur teilweise demethyliert und oxydiert. Sie werden hauptsächlich als Methylharnsäure oder als Methylxanthine ausgeschieden. Bereits 1957 wurde nachgewiesen, dass beim Menschen Theophyllin in erster Linie in 1,3-Dimethyl-Harnsäure umgewandelt wird: Theobromin wird sowohl als 3-Methyl- wie auch als 7-Methylxanthin ausgeschieden: Koffein wird als 1-Methyl-Harnsäure und 1-Methylxanthin in relativ gleichen Mengen ausgeschieden. Ungefähr 10% dieses Alkaloids werden unverändert ausgeschieden. Da keines der Xanthine vollständig demethyliert wird, nimmt die Ausscheidung der wirklichen Harnsäure nicht zu. Dieser wichtige Umstand bedeutet, dass die Xanthine bei Gichtkranken nicht kontraindiziert sind».

3. Diabetes mellitus

Die Werbung für koffeinfreien Kaffee – auf Grund von Vermutungen eines amerikanischen Wissenschaftlers – hat leider dazu beigetragen, Diabetiker hinsichtlich der erlaubten Menge ihres Kaffeekonsums zu verunsichern [6]. Die Werbung besagt unter anderem, dass viele Diabetiker der Meinung seien, Kaffee wäre für sie unschädlich, «obwohl durch neue wissenschaftliche Erkenntnisse diese Auffassung doch recht fragwürdig geworden ist». Es wird behauptet, dass «sich gerade der Koffeingehalt negativ auf den Stoffwechsel des Diabetikers auswirkt». Mit dieser Werbung sollen Millionen von Diabetikern unter anderem davor gewarnt werden, dass «durch Koffein beim Diabetiker die Betazellen des Pankreas zusätzlich belastet» würden. Ausserdem könne Koffein die Symptome des Diabetes «verstärken». «Schon 2 Tassen normal starken koffeinhaltigen Kaffees lassen den Blutzuckerspiegel und die Säurewerte des Blutserums beim Diabetiker deutlich ansteigen.»

Nachdem wir in der Evans County-Studie [2] statistisch nicht-signifikante Unterschiede zwischen starken Kaffeekonsumenten und Personen ohne oder mit nur geringem Kaffeekonsum beobachtet hatten (Männer mit starkem Kaffeekonsum durchschnittlich 100 mg% Nüchternblutzucker, Männer mit geringem oder keinem Kaffeekonsum 93 mg%; Frauen mit hohem Kaffeekonsum 98 mg% und Frauen mit geringem oder keinem Kaffeekonsum 94 mg%), stellten wir darüber hinaus fest, dass starke Kaffeekonsumenten ebenso wie Nicht-Kaffeekonsumenten *gleich häufig Diabetes mellitus* hatten. Wir schliessen uns daher der Meinung von MEINERTZ an (persönliche Mitteilung 1973): «Die Frage, ob Koffein für Diabetiker schädlich ist, lässt sich eindeutig verneinen. Es ist zwar richtig, dass Koffein die Insulinsekretion aus der Bauchspeicheldrüse fördert, ein schädlicher Effekt – im Sinne einer Erschöpfung der Insulinproduktion – wurde aber niemals beobachtet. Im Grunde ist die Wirkung des Koffeins viel eher vergleichbar mit der von Medikamenten (wie z. B. Sulfonamid-Präparaten), die an Diabetiker verabreicht werden, um eine Stoffwechselbesserung einzuleiten. Dies kann zwar nicht bedeuten, dass man Koffein zur Behandlung des Diabetes einsetzt (dazu ist der Effekt zu schwach); überhaupt kein Grund besteht aber, koffeinhaltigen Kaffee zu verbieten. Nachdem uns Ärzten leider bei der Diätbehandlung des Diabetes in vielfacher Hinsicht die Hände gebunden sind und wir manches, was die Patienten mögen, verbieten oder einschränken müssen, sollte man die Verbotsliste nicht unnötig auf Genussmittel erweitern, gegen die nichts einzuwenden ist.»

4. Myokardinfarkt

Unter dem Thema Kaffeekonsum und akuter Myokardinfarkt erschien 1972 ein Bericht [7] des Boston Collaborative Drug Surveillance-Program, wonach kaffeetrinkende Frauen ein 4mal höheres Risiko für Herzinfarkt als Frauen, die keinen Kaffee trinken, haben sollten und Kettenraucher ein niedrigeres Infarktrisiko als starke Kaffeekonsumenten hätten! 1973 wurden von derselben Forschungsgruppe [8] weitere Resultate veröffentlicht: Auf Grund einer Befragung von 440 Herzinfarktpatienten und über 12 000 hospitalisierten Patienten als Kontrollgruppe ergaben sich gegenüber Nicht-Kaffeekonsumenten «ein um 60% höheres Infarktrisiko bei täglichem Genuss von 1 bis 5 Tassen Kaffee und ein um 120% höheres Risiko bei über 6 Tassen pro Tag». Überraschenderweise bestand keine Assoziation zwischen Teegenuss und Infarkt (1 Tasse starken Tees enthält bekanntlich ebenso wie die eines starken Kaffees 120–150 mg Koffein!). Auch diese Studie ist retrospektiv und beschränkt sich auf hospitalisierte Patienten; da vor der Spitalaufnahme am Herzinfarkt verstorbene Patienten nicht erfasst werden konnten, fragt sich, inwieweit die Infarktgruppe bei der 30%igen Letalität dieser Erkrankung vor der Spitalaufnahme repräsentativ ist. Ausserdem sind auch die kurz nach der Hospitalisierung verstorbenen Patienten nicht befragt worden. Zweifel sind auch an der Repräsentativität der Kontrollgruppe angebracht. Wie die Autoren der Framingham-Studie [9] in ihrem Editorial bemerken, sind diese Kontrollpatienten aus verschiedenen medizinischen und chirurgischen Abteilungen zahlreicher Kliniken sicher nicht mit der Allgemeinbevölkerung vergleichbar. Potentiell müssen darunter ungewöhnlich viele Leute sein, die aus irgendeinem Grunde Kaffee mieden, sei es freiwillig oder auf Anraten eines Arztes. Die Bostoner Kollegen [7] formulieren sehr vorsichtig, dass auf Grund ihrer Ergebnisse eine kausale Erklärung der Assoziation Kaffeegenuss–Myokardinfarkt nicht gerechtfertigt ist. Die Resultate dieser retrospektiven Untersuchungen stehen in eindeutigem Gegensatz zu denjenigen der prospektiven Studien von Chicago [10] und Framingham [9]. PAUL hat in einer zweiten Publikation [11] zugeben müssen, dass nicht der Kaffeekonsum per se für die höhere Infarktrate unter Kaffeetrinkern verantwortlich sei, sondern der Zigarettenkonsum. In der Gothenburg-Studie – ebenfalls prospektiv über einen 8-Jahres-Zeit-

[illegible] – wurde kein Zusammenhang zwischen Kaffeetrinkgewohnheiten und Herzinfarkt beobachtet (WILHELMSEN und TIBBLIN, im Druck 1973). Eine andere retrospektive Studie [2] verglich die Infarktgruppe mit der *gesunden Bevölkerung*, die als *Kontrollgruppe* diente. Es fand sich überhaupt kein Unterschied in bezug auf den Kaffeekonsum zwischen Herzinfarktpatienten und Gesunden.

Schlussfolgerung: Vom ärztlichen Standpunkt aus besteht weder bei Hypertonikern, Gichtpatienten und Diabetikern noch in der primären oder sekundären Prophylaxe des Herzinfarktes ein Grund, den Kaffeekonsum der Patienten einzuschränken.

1. DAWBER T. R., KANNEL W. B., KAGAN A., DONABEDIAN R. K., MCNAMARA P. M. und PEARSON G.: Environmental factors in hypertension. In: Epidemiology of hypertension, S. 225–288. Grune & Stratton, New York 1967.
2. HEYDEN S., BARTEL A., CASSEL J. C., HAMES C. G., TYROLER H. A. und MEIER R.: Kaffeekonsum, Gefässerkrankungen und Risikofaktoren in der Evans-County/Georgia-Studie. Z. Ernährungswiss., Suppl. 14, S. 1–10 (1972).
3. HAUSCHILD F. und GÖRISCH V.: Einführung in die Pharmakologie und Arzneiverordnungslehre, S. 317–319. Thieme, Leipzig 1967.
4. KUSCHINSKY G. und LÜLLMANN H.: Kurzes Lehrbuch der Pharmakologie, 5. Aufl. Georg Thieme Verlag, Stuttgart 1972.
5. GOODMAN L. S. und GILMAN A.: The pharmacological basis of therapeutics, 4th Ed. MacMillan Company, New York 1970.
6. HAG AG, Bremen: Kaffee für Diabetiker? In: Bircher-Benner, Handbuch Nr. 14 (1973).
7. Boston Collaborative Drug Surveillance Program: Coffee drinking and acute myocardial infarction. Lancet 1972, II, 1278–1281.
8. JICK H., MILTTINEN O. S., NEFF R. K., SHAPIRO S., HEINONEN O. P. und STONE D.: Coffee and myocardial infarction. New Engl. J. Med. 289, 63–67 (1973).
9. KANNEL W. B. und DAWBER T. R.: Coffee and coronary disease. Editorial. New Engl. J. Med. 289, 100–101 (1973).
10. PAUL O., MACMILLAN A., MCKEAN H. und PARK E.: Sucrose intake and coronary heart disease. Lancet 1968, II, 1049.
11. PAUL O.: Stimulants and coronaries. Postgrad. Med. 44, 196 (1968).

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COFFEE DRINKING, SMOKING, POLLUTION, AND CARDIOVASCULAR DISEASE: A PROBLEM OF SELF-SELECTION

SIR,—Risk of certain cardiovascular diseases has been found associated with cigarette smoking,[1] coffee drinking,[2,3] diabetes mellitus,[4,5] and the use of oral anti-diabetic drugs.[4,5] Smokers tend to be coffee drinkers,[2,3,6] and male smokers, including diabetics, tend to display higher blood-glucose levels than controls.[6,7]

Two hypotheses are commonly advanced to explain the association between smoking and chronic diseases: (a) smoking causes chronic diseases, and (b) smoking and chronic diseases associated with smoking are influenced by a common cause—the individual constitution or genotype.[6-9] Comparable hypotheses, (c) and (d), respectively, may explain the association between coffee drinking and chronic diseases.

Tobacco smoking may often be an effect or symptom of deficient glucose metabolic bioenergetics of constitutional origin,[6,7,9] as may coffee consumption and, in extreme cases, the use of anti-diabetic drugs.[4,5] Based on considerations of evolutionary biology, tobacco smoking may be a self-selected behavioural regulator of physiological homœostasis—i.e., an ethological homœostatic process,[9] with nicotine functioning to alleviate metabolic dysoxidoses.[6,7,9,10] A common basis underlying the coffee-consumption/cigarette-smoking association may involve biogenic amine physiology.[6,7,9] Nicotine induces adrenal and other cellular release of catecholamines[6,7,11] with consequent increases in metabolic-rate[6,7,9] and drug metabolism.[7] Caffeine is a monoamine-oxidase (M.A.O.) inhibitor,[6] and theophylline induces adrenal release of catecholamines.[11] Nicotine,[12] caffeine,[13] and theophylline[13] pass the blood/brain barrier readily and so can affect cerebral-nervous-system physiology directly.

The assertion[14] that "cocoa drinking is innocuous or mildly beneficial, tea beneficial, and coffee harmful to the heart of a person with healthy coronary vessels" must be considered speculative. It appears to be based on (a) statistical association and (b) a presumption that "reasonable" judgment is equivalent to truth. Such a method is scientifically invalid. Applying this "method" to data of Hrubec[3] on "angina", one might "deduce", improperly, that coffee is "harmful to the heart" for those who smoke more than 31 cigarettes per day, but "beneficial to the heart" for those who smoke 1–30 a day.

Alternatively, if tobacco smoking and coffee consumption are both largely self-selected[7,9,15] by influences of ethological homœostasis,[6,9,16] then metabolic dysoxidoses involving biogenic amine physiology[6,7,9] may be alleviated by caffeine, nicotine, or both. Their effects may be synergistic since the M.A.O. inhibitory activity of caffeine can reduce inactivation of biogenic amines released by nicotine. Smokers of more than 30 cigarettes a day are a self-selected subgroup[16] which, on the average, may have more severe metabolic dysoxidoses than the self-selected subgroup that smokes 1–30 a day.[3] Caffeine and nicotine jointly may alleviate the metabolic problems of the latter group more readily than those of the former. These observations[3] seem compatible with constitutional hypotheses (b) and (d), above, rather than with hypotheses (a) and (c).

The degree of non-alleviation of metabolic dysoxidoses[9,10] may be proportional to the risk of accumulation of somatic mutations.[6-9] Multiple regression analyses showed atmospheric levels of SO_2 and NO_2 significantly correlated positively with mortality-rates for arteriosclerotic heart-disease (I.S.C. 420) in metropolitan populations of the United States.[6-9] Acute myocardial infarction (I.S.C. 420.1) is subsumed in this category.

A self-selected subgroup of people may exist which is constitutionally more likely to (a) drink coffee, (b) incur acute myocardial infarction and "angina", and (c) be at increased risk of accumulating somatic mutations than non-coffee-drinking controls. This subgroup may also be more likely to smoke cigarettes than the controls.

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1. The Health Consequences of Smoking. U.S. Public Health Service. Washington, D.C., 1971.
2. Vessey, M. P. (for Boston Collaborative Drug Surveillance Program). *Lancet*, 1972, ii, 1278; *ibid.* 1973, i, 314.
3. Hrubec, Z. *ibid.* p. 548.
4. Hadden, D. R., Montgomery, D. A. D., Weaver, J. A. *ibid.* 1972, i, 335, 537.
5. Boyle, D., Bhatia, S. K., Hadden, D. R., Montgomery, D. A. D., Weaver, J. A. *ibid.* p. 338.
6. Hickey, R. J., Harner, E. B. *in* Smoking Behavior: Motives and Incentives (edited by W. L. Dunn, Jr.). Washington, D.C., 1973.
7. Hickey, R. J., Clelland, R. C., Harner, E. B. *Lancet*, 1973, i, 270.
8. Hickey, R. J., Harner, E. B., Clelland, R. C. *ibid.* 1972, ii, 1362.
9. Hickey, R. J., Harner, E. B., Clelland, R. C., Boyce, D. E. *Naturwissenschaften* (in the press).
10. Pentschew, A., Garro, F. *Acta neuropath., Berlin*, 1966, 6, 266.
11. Poisner, A. M. *Biochem. Pharmacol.* 1973, 22, 469.
12. Oldendorf, W. H. *Trans. Am. neurol. Ass.* 1971, 96, 46.
13. Thithipandha, A., Maling, H. M., Gilette, J. R. *Proc. Soc. exp. Biol. Med.* 1972, 139, 582.
14. Rajapka, D. A. *Lancet*, 1973, i, 313.

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Alcohol and Caffeine in Choice-Discrimination Tests in Rats. (26878)

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It is a popular belief that caffeine is an effective antagonist to alcohol. This is undoubtedly based on the generalization that alcohol is a central depressent while caffeine exerts a stimulant action centrally. Despite the apparent "stimulation" from alcohol which is evident in some persons, biological scientists agree that, if classification is essential, ethanol must be categorized as a depressant.

However, both hypnotics and tranquilizers are also central depressants. The mechanism of action, herein, as well as end results are very different and terminology such as stimulant or depressant drug is becoming meaningless.

Popular usage of the term tranquilizer often suggests that alcohol has such properties. This has been challenged since a tranquilizer should reduce anxiety without greatly obtunding other behavioral responsiveness (1). We have shown that alcohol will diminish anxiety but does so only at the expense of various parameters of performance and behavior(2,3,5).

This study was begun in order to measure the effect of alcohol-caffeine combinations on performance in the rat. It was anticipated that caffeine might reverse some of the effects of alcohol since this is so commonly reported.

Materials and methods. The experimental technique employed a shock-avoidance box (Fig. 1) in which a choice must be made for escape(1,2,3,4). The floor of the main compartment (A) consists of stainless steel rods. A warning light (L) serves as a conditioning stimulus and is presented 5 seconds before a shock is delivered *via* the grid flooring. The door (D) is lifted simultaneously with the warning light to expose the escape compartments (B) and (C). If the rat fails to escape, 5 seconds later he receives a 5-second shock delivered by the grid floor. The rat may escape to a *safe* and darkened area (B) with insulated non-shock floor, or to an *unsafe* illuminated area (C) with a grid floor that will deliver shock.

The only animals used in this study were those that had learned to respond with 100% accuracy to the "anxiety-producing" warning light by escaping to the *safe* area (B), thus avoiding shock. When the rat observes and reacts to the warning light, we consider this to be a simple conditioned avoidance response (CR). If the rat fails to leave the main compartment during the 5 seconds in which the light is on, a shock of 15v A.C. from a relatively low resistance power supply is applied to the grid for 5 seconds. If this is effective and the animal leaves the main compartment, this is recorded as an unconditioned avoidance response (UR). With our apparatus, the rat must make a decision-choice (discrimination) of the correct compartment into which he must flee. We recorded errors in discrimination as incorrect choices of compartment after a stimulus of light or of shock.

Weanling male rats were trained 3 times daily and at each testing period received 3 trials. With this 9-trial daily testing period, 90 to 95% of the animals learned to escape properly with 100% accuracy in 4 days.

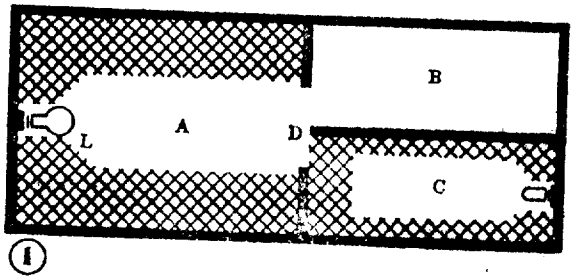
All drugs were administered intraperitoneally. Alcohol was administered as a 5% solution. Caffeine was administered as the citrate salt but calculated as 50, 100 or 150 mg/kg of body weight of the base.

After administration of the alcohol, caffeine or alcohol-caffeine combinations, the animals were tested 3 times every 15 minutes for 4 hours thereafter. Dosage levels were: 50 mg/kg, 100 mg/kg, and 150 mg/kg of caffeine, 1 g/kg of ethanol, and 1 g/kg of ethanol combined with 50, 100, or 150 mg/kg of caffeine. A control group of rats received only saline injections. Each of the total of 90 rats was used for only one 4 hour test and discarded at the end of the test period. The dosage of ethanol was selected because previous reports from our laboratory indi-

cated that this induces only a moderate effect on diminishment of performance(3). Larger doses may cause gross immobility of the animal. Preliminary studies indicated that 5, 10 or 25 mg/kg of caffeine effected no significant change in responsiveness in the animals. Therefore, 50 mg/kg of caffeine was used as the low dosage level.

Results. Total scores for the animals for each hour are represented graphically in Fig. 2, 3, 4. The control group of 10 rats continued to respond correctly, *i.e.*, no suppression of total conditioned responses. A small dose of caffeine caused no significant reduction in conditioned responses. The intermediate dose (100 mg/kg) effected a moderate reduction in conditioned responses for the first 2 hours only. A large dose (150 mg/kg) of caffeine caused a marked reduction in CR which lasted longer than the 4 hour period. Alcohol alone (1 g/kg) caused a slight (5%) reduction of CR during the first hour. However, when alcohol was combined with caffeine, the rats were markedly affected in that a greater reduction in conditioned responses resulted than could be explained by addition of the 2 drugs. There was no drug antagonism demonstrated by this "stimulant-depressant" combination. Thus, potentiation was most evident when an intermediate dosage of caffeine was combined with alcohol.

A like set of results was obtained for the



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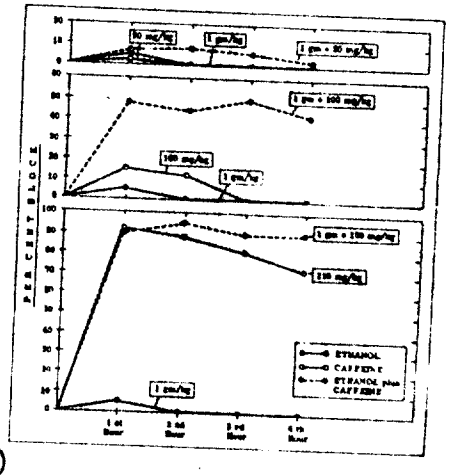

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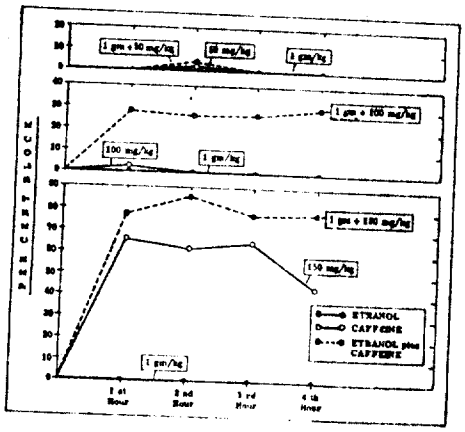

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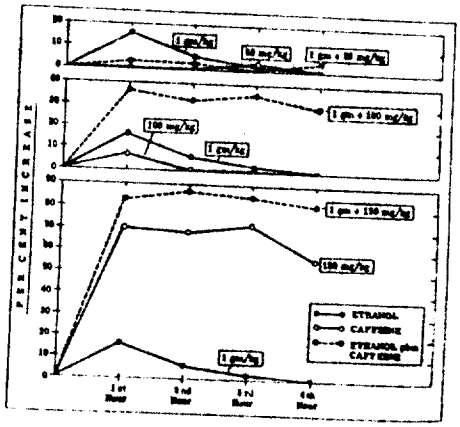

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FIG. 1. Apparatus and training method. A, main compartment: warning light, shocking grid floor. B, safe escape area: not illuminated, insulated floor. C, unsafe escape area: illuminated, shocking grid floor. D, Guillotine-type door. L, warning light.

FIG. 2. Suppression of conditioned responses in rats after alcohol, caffeine and alcohol-caffeine combinations.

FIG. 3. Suppression of unconditioned responses in rats after caffeine, alcohol and caffeine-alcohol combinations.

FIG. 4. Increase in errors in choice-discrimination in rats after alcohol, caffeine and alcohol-caffeine combinations.

unconditioned response (Fig. 2). Once again alcohol-caffeine combinations gave greater depression of responses than simple addition can explain.

Similarly, an increase in errors of choice of exits (discrimination) was observed after caffeine and alcohol were combined (Fig. 4). The rats were not noticeably stimulated by 50 or 100 mg/kg of caffeine or depressed by the alcohol alone. However, when 100 mg/kg of caffeine and 1 g/kg of ethanol were administered to the animals, an obvious depressed state resulted. The animals receiving this combination were able to respond to the unconditioned shocking stimulus but did so in a confused manner and with a large number of errors. This potentiation was also evident in the animals' receiving larger doses (150 mg/kg) of caffeine.

Discussion. An intraperitoneal injection of 1 g/kg of ethanol will effect a peak blood level in rats of approximately 90 mg% in 15 minutes and this will have largely disappeared from the blood within 2 hours(3). In the present study the effect of alcohol had disappeared by the second hour. This dose of alcohol did not produce behavioral changes such as drowsiness, reduction in activity or agressiveness. Caffeine in a dosage of 100 mg/kg i.p. altered behavior which returned to normal at the end of 2 hours. With this dosage of caffeine, some of the animals showed some hyperexcitability when handled.

The above dose of alcohol (1 g/kg) combined with caffeine (100 mg/kg) produced an effect in behavioral responses which was still present at the end of 4 hours. These animals were grossly depressed. Activity was not measured but was obviously reduced at the end of 4 hours, although the animals that received either drug alone appeared to be unaffected. The degree of gross depression seems to correlate with the reduction in behavioral responsiveness measured. It is apparent from the data that alcohol altered the behavior in such a manner that recovery was not complete although blood levels might indicate otherwise. There is evidence that recovery had scarcely begun when zero blood levels of alcohol had been reached.

The same potentiation is apparent when higher dosages (150 mg/kg) of caffeine were used but this concentration of the alkaloid is grossly depressant by itself.

It was not evident from our studies that caffeine antagonized alcohol. Rather it appears that caffeine potentiates the slight depression of conditioned and unconditioned responses due to this dosage of alcohol as well as markedly increasing errors in discrimination.

Studies in human subjects are in progress to test the hypothesis that alcohol produces behavioral degeneration which remains long after the alcohol has disappeared from the blood and that certain drugs, including stimulants, may exaggerate this effect.

Summary. A series of rats received alcohol, caffeine, or alcohol-caffeine combinations. Conditioned and unconditioned responses as well as erors in choice (discrimination) were measured. No significant changes were noted in total conditioned or unconditioned responsiveness with small doses of caffeine (50 mg/kg) or with a dosage of 1 g/kg of ethanol. By all measurements, as well as observation, caffeine potentiated the depressive effect of alcohol long after the alcohol had disappeared from the blood. This was most marked with intermediate (100 mg/kg) or with high (150 mg/kg) dosages of caffeine. In no instance did caffeine antagonize the effects of alcohol.

1. Hughes, F. W., Kopmann, E., *Arch. de Pharmacodyn. et de Therap.*, 1960, v126, 158.

2. Kopmann, E., Hughes, F. W., *A.M.A. Arch. Gen. Psychiat.*, 1959, v1, 7.

3. Hughes, F. W., Rountree, C. B., *Arch de Pharmacodyn. et de Therap.*, 1961, in press.

4. Kopmann, E., Hughes, F. W., *Experientia*, 1958, v14, 8.

5. Forney, R. B., Hughes, F. W., *J. Psychol.*, 1961, v52, 185.

Received June 26, 1961. P.S.E.B.M., 1961, v108.

Lancet 2:1278-1281. 1972.

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COFFEE DRINKING AND ACUTE MYOCARDIAL INFARCTION

Report from the Boston Collaborative Drug Surveillance Program*

Summary The daily hot beverage intake before admission to hospital of 276 patients with acute myocardial infarction has been compared with that of 1104 matched control patients with other diseases. No significant difference in the quantity of tea ingested was found between the two groups, but the patients with acute myocardial infarction were found to drink appreciably more coffee than the matched controls. This difference could not be explained in terms of cigarette smoking. It is suggested that the possible role of coffee drinking in the ætiology of acute myocardial infarction requires re-evaluation.

Introduction

A CONSISTENT finding in the Boston Collaborative Drug Surveillance Program (B.C.D.S.P.) [1] has been that patients discharged from hospital with a diagnosis of acute myocardial infarction drink more coffee on average than patients discharged without that diagnosis. We report here an evaluation of this finding.

Materials and Methods

In the B.C.D.S.P., patients are asked, on admission to hospital, whether they normally drink zero, one to five, or more than five cups of coffee daily; patients are similarly questioned about tea drinking. Data of this type were obtained from 316 patients with a discharge diagnosis of acute myocardial infarction. These patients had been admitted to one or other of eight different hospitals (four in the United States, two in Canada, one in Israel, and one in New Zealand). Known alcoholics and non-whites were excluded, reducing the number of patients with acute myocardial infarction to 300. Comparisons were then made between these patients and almost 7000 other patients with other diagnoses. This procedure showed that age, sex, and hospital were important confounding variables which would have to be controlled in any further analyses and it was decided to attempt to match each of the 300 patients with acute myocardial infarction with 4 control patients without that diagnosis, taking into account decade of age, sex, and hospital. 276 (92%) of the patients were

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The report was written by Dr. Martin P. Vessey, University of Oxford.

matched in this way. The remaining 24 patients, who did not differ in any important way from the other 276, have been excluded from further analysis.

Of the 276 successfully matched patients with acute myocardial infarction, 49% were admitted to hospitals in the United States, 70% were male, 43% were less than 60 years of age, 17% were recorded as being diabetic, and 8% were recorded as being hypertensive. Unfortunately, no information is available as to how many of the patients had had a previous myocardial infarct.

A wide variety of diagnoses was represented among the 1104 matched controls: 10% had some form of cancer, 15% had a chronic respiratory disease, 14% were recorded as having diabetes, and 11% were recorded as being hypertensive. Most important of all, however, 25% had as one of their discharge diagnoses (up to five are recorded by the B.C.D.S.P.) some form of ischæmic heart-disease other than acute myocardial infarction—most commonly described as chronic ischæmic heart-disease or arteriosclerotic heart-disease. This finding immediately raised questions as to the appropriateness of the control series. Comparisons were therefore made between those controls with a discharge diagnosis of some form of ischæmic heart-disease other than acute myocardial infarction and those without such a diagnosis. No suggestive differences were found between the two groups with respect to coffee or tea consumption. In regard to smoking, there was a modest excess of ex-smokers in the former group. It was concluded that it was reasonable to retain the complete control series for comparison with the patients with acute myocardial infarction.

TABLE I—COFFEE AND TEA CONSUMPTION AMONG PATIENTS WITH ACUTE MYOCARDIAL INFARCTION AND MATCHED CONTROL PATIENTS

Group	Consumption (cups/day)			
	0	1-5	6+	Total
Coffee:				
M.I.	48	188	40	276
Controls	256	748	100	1104
Risk ratio	*1·00*	*1·34*	*2·13*	
Tea:				
M.I.	119	146	11	276
Controls	433	610	61	1104
Risk ratio	*1·00*	*0·87*	*0·66*	

M.I. = Myocardial infarction.

Test for linear trend: Coffee consumption $\chi^2_{(1)} = 8{\cdot}9$ ($P = 0{\cdot}003$);

Tea consumption $\chi^2_{(1)} = 2{\cdot}0$ ($P = 0{\cdot}16$).

Results

Patients with acute myocardial infarction drank more coffee than did the matched controls ($P < 0{\cdot}01$) (table I). In regard to tea consumption the trend is, if anything, in the opposite direction, but this difference is not statistically significant. Coffee and tea consumption are, of course, negatively correlated, and the intake of tea was much higher among the patients admitted to hospitals in Canada, Israel, and New Zealand than among the patients admitted to the hospitals in the United States. However, examination of the data classified by both coffee and tea intake, for the American and non-American hospitals separately, provided no evidence of any consistent association between tea drinking and acute myocardial infarction. For this reason, the remainder of the analysis is concerned only with coffee ingestion.

TABLE II—COFFEE CONSUMED AMONG PATIENTS WITH ACUTE MYOCARDIAL INFARCTION AND MATCHED CONTROL PATIENTS BY SEX, AGE, AND HOSPITAL

—	Coffee consumption (cups/day)			
	0	1-5	6+	Total
Males:				
M.I.	30	132	31	193
Controls	174	508	90	772
Risk ratio	*1·00*	*1·51*	*2·00*	..
Females:				
M.I.	18	56	9	83
Controls	82	240	10	332
Risk ratio	*1·00*	*1·06*	*4·10*	..
Age < 59 yr.:				
M.I.	18	75	26	119
Controls	108	295	73	476
Risk ratio	*1·00*	*1·53*	*2·14*	..
Age 60+ yr.:				
M.I.	30	113	14	157
Controls	148	453	27	628
Risk ratio	*1·00*	*1·23*	*2·56*	..
U.S. hospitals:				
M.I.	18	91	27	136
Controls	97	391	56	544
Risk ratio	*1·00*	*1·25*	*2·60*	..
Non-U.S. hospitals:				
M.I.	30	97	13	140
Controls	159	357	44	560
Risk ratio	*1·00*	*1·44*	*1·57*	..

Table II shows that the association between coffee ingestion and acute myocardial infarction is present in males and females, in young (up to 59 years) and old (60 or more years), and in the American hospitals and the non-American hospitals. The association was also found to hold in those with and those without diabetes and in those with and those without hypertension.

Coffee ingestion and cigarette smoking were strongly correlated. However, the association between coffee ingestion and acute myocardial infarction

TABLE III—PATIENTS WITH ACUTE MYOCARDIAL INFARCTION AND CONTROL PATIENTS IN RELATION TO CURRENT SMOKING STATUS AND COFFEE CONSUMPTION

Group	Coffee consumption (cups/day)			
	0	1-5	6+	Total
Non-smoker:				
M.I.	16	55	5	76
Controls	104	250	8	362
Risk ratio	*1·00*	*1·43*	*4·06*	
Up to 1 pack cigarettes per day:				
M.I.	9	52	12	73
Controls	53	185	29	267
Risk ratio	*1·10*	*1·83*	*2·69*	
More than 1 pack cigarettes per day:				
M.I.	8	24	14	46
Controls	28	75	29	132
Risk ratio	*1·86*	*2·08*	*3·14*	
Ex-smokers:				
M.I.	12	38	6	56
Controls	48	153	16	217
Risk ratio	*1·63*	*1·61*	*2·44*	
Smoking of variable numbers of cigarettes, pipes, cigars:				
M.I.	3	19	3	25
Controls	23	85	18	126
Risk ratio	*0·85*	*1·45*	*1·08*	

TABLE IV—MEAN INTAKE OF COFFEE AND TEA BY PATIENTS WITH AND WITHOUT CORONARY-ARTERY DISEASE: REVIEW OF THE LITERATURE

Series	Country	Form of coronary-artery disease	Size of series		Mean intake (cups per day) of:			
					Coffee		Tea	
			Cases	Controls	Cases	Controls	Cases	Controls
Paul et al.*[4]	U.S.A.	Angina, myocardial infarction, or sudden death	54	1108	4·2	3·5	..	..
Yudkin and colleagues[2,3]	England	Myocardial infarction	40	58	0·8	1·4	7·7	5·6
Little et al.[9]	Canada	Myocardial infarction	86	84	1·3	1·3	3·2	3·6
Howell and Wilson[10]	England	"Definite" or "possible" ischæmic heart-disease	170	1158	1·0	1·1	6·0	5·7

* Mean intake of coffee for cases and controls calculated from frequency distributions provided by Paul et al.

could not be explained in terms of cigarette smoking (table III): indeed, the association between coffee ingestion and acute myocardial infarction seems stronger than the association between cigarette smoking and the disease. The control series, however, would be expected to underestimate the relation between cigarette smoking and acute myocardial infarction because 15% of the controls had chronic respiratory disease and almost 2% had carcinoma of the bronchus, diseases themselves strongly associated with smoking.

Discussion

The data suggest that people drinking more than five cups of coffee per day have about twice as great a risk of having an acute myocardial infarction as people drinking no coffee at all. There are, however, many possible explanations for the findings other than a direct causal relationship. Quite apart from all the well-known selective biases in retrospective studies of people admitted to hospital, an unknown proportion of the patients with acute myocardial infarction had had a previous attack of the disease, and these patients might have increased their consumption of coffee as a reaction to the stress of having had a heart attack. In one respect, however, the data have particular strength. Information about coffee and tea ingestion was collected as part of a routine inquiry, and the interviewers were unaware of any particular interest in patients with acute myocardial infarction.

The absence of any association between coffee ingestion and forms of ischæmic heart-disease other than acute myocardial infarction should be interpreted with caution. The B.C.D.S.P. records up to five discharge diagnoses for each patient, and inclusion of terms such as chronic ischæmic heart-disease or arteriosclerotic heart-disease in the list is so common (25% in the controls) and often on such slender evidence, that the specificity of the diagnosis in many patients is doubtful. By contrast, evidence in support of the diagnosis acute myocardial infarction when made in hospital has been found to be good in a further study to be reported by the B.C.D.S.P.

A lot of attention has been paid to a possible association between sucrose intake and coronary-artery disease, especially sucrose taken in tea and coffee. Yudkin and his colleagues[2,3] found that men with myocardial infarction had a much higher intake of sucrose and a higher intake of hot beverages than did controls. These workers concluded that the main association was between sucrose intake and the disease, the hot beverages merely acting as a vehicle for the sucrose. Other epidemiological studies of the possible association between sucrose intake and coronary-artery disease have, however, been essentially negative.[4-12]

Unfortunately, we have no information on sucrose intake in the B.C.D.S.P. patients. None the less, an association between coffee ingestion and acute myocardial infarction might be considered to support Yudkin's hypothesis, but there is one major discrepancy—we could find no association between tea drinking and the disease, and such an association would be expected if sucrose intake was the underlying explanation of our results. Rather, the findings in the present study suggest that coffee itself is important.

The published investigations concerned with sucrose intake and coronary-artery disease include little information about coffee drinking; of those that do provide data (see table IV), only the one by Paul et al.[4] supports the hypothesis that coffee intake might be related to coronary-artery disease, though Paul's group later showed that most of the apparent association between coffee drinking and coronary-artery disease could be explained by cigarette smoking.[5]

However, apart from the study by Paul et al.[4,5] all the investigations shown in table IV were done in countries where tea drinking is more common than coffee drinking. Furthermore, most of the series are small, two include patients with forms of ischæmic heart-disease other than acute myocardial infarction (which show no relationship with coffee ingestion in our data), and, except in the study by Paul et al.,[4] only mean coffee intake was reported. In our opinion these investigations do not rule out the possibility that a heavy intake of coffee might be related to the risk of acute myocardial infarction. Further studies are needed in countries, such as the United States, where large quantities of coffee are drunk. The B.C.D.S.P. is now collecting information about the intake of hot beverages from a much larger group of patients with acute myocardial infarction and suitable control subjects.

If people who drink a lot of coffee are indeed at an increased risk of acute myocardial infarction, how might this finding be explained? Coffee drinking might merely be a feature of the striving, competitive personality which some workers believe to be characteristic of those prone to coronary-artery disease.[13] It is also possible, however, that coffee might have some specific injurious effect. This effect cannot be attributed to caffeine, because both tea and coffee contain 100–150 mg. caffeine per cup. Little et al.[14] found that in a group of 85 men who had survived myocardial infarction, there was a positive correlation between

the daily intake of coffee and the serum-cholesterol level. No such association was found when the daily intake of tea was considered. It is possible, therefore, that coffee might exert an effect via the serum-lipids.

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REFERENCES

1. Jick, H., Miettinen, O. S., Shapiro, S., et al. *J. Am. med. Ass.* 1970, **213**, 1455.
2. Yudkin, J., Roddy, J. *Lancet*, 1964, ii, 6.
3. Yudkin, J., Morland, J. *Am. J. clin. Nutr.* 1967, **20**, 503.
4. Paul, O., Lepper, M. H., Phelan, W. H., Dupertuis, G. W., MacMillan, A., McKean, H., Park, H. *Circulation*, 1963, **28**, 20.
5. Paul, O., MacMillan, A., McKean, H., Park, H. *Lancet*, 1968, ii, 1049.
6. Little, J. A., Shanoff, H. M., Csima, A., Redmond, S. E., Yano, R. *ibid.* 1965, i, 933.
7. Papp, O. A., Padilla, L., Johnson, A. L. *ibid.* 1965, ii, 259.
8. Finegan, A., Hickey, N., Maurer, B., Mulcahy, R. *Am. J. clin. Nutr.* 1968, **21**, 143.
9. Burns-Cox, C. J., Doll, R., Ball, K. P. *Br. Heart J.* 1969, **31**, 485.
10. Howell, R. W., Wilson, D. G. *Br. med. J.* 1969, iii, 145.
11. Medical Research Council Working Party, *Lancet*, 1970, ii, 1265.
12. Elwood, P. C., Waters, W. E., Moore, S., Sweetnam, P. *ibid.* 1970, i, 1014.
13. *Br. med. J.* 1969, i, 1.
14. Little, J. A., Shanoff, H. M., Csima, A., Yano, R. *Lancet*, 1966, i, 732.

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COFFEE AND MYOCARDIAL INFARCTION

HERSHEL JICK, M.D., OLLI S. MIETTINEN, M.D., PH.D., RAYMOND K. NEFF, M.S., SAMUEL SHAPIRO, M.D., OLLI P. HEINONEN, M.D., AND DENNIS SLONE, M.D.

A Report from the Boston Collaborative Drug Surveillance Program, Boston University Medical Center

Abstract A positive association between coffee consumption and acute myocardial infarction was confirmed by analyses of data from a multipurpose survey of 12,759 hospitalized patients, including 440 with a diagnosis of acute myocardial infarction. As compared with those who drink no coffee, the risks of infarction among those drinking one to five and six or more cups of coffee per day are estimated to be increased by 60 and 120 per cent, respectively. This association could not be attributed to confounding by age, sex, past coronary heart disease, hypertension, congestive heart failure, obesity, diabetes, smoking or occupation, nor could it be explained by the use of sugar with coffee. There was no positive association between tea drinking and acute myocardial infarction. (N Engl J Med 289:63-67, 1973)

A POSITIVE association between coffee consumption and acute myocardial infarction was recently reported from the Boston Collaborative Drug Surveillance Program.[1] Age and sex were controlled by matching, and smoking was taken into account in the analysis. Data on the use of sugar with coffee were not obtained. Nevertheless, sugar as an explanation of the association seemed to be inconsistent with the observation that the use of tea showed, if anything, a negative association with acute myocardial infarction. The possibility that the high level of coffee consumption among cases of myocardial infarction is a consequence of previous coronary heart disease was also considered.

The present report concerns further evaluation of the purported association on the basis of a completely separate body of data. This new series is larger than the previous one, and it includes data for direct evaluation of possible confounding by the intake of sugar and by previously diagnosed coronary heart disease.

SUBJECTS AND METHODS

The present study is based on a special survey, carried out in 24 Boston area hospitals during January to September, 1972. The aim of the survey was to relate histories of use of a broad range of drugs to various diseases in a search for etiologic or preventive connections. Besides drugs, routine use of tobacco, coffee, tea and the addition of sugar to coffee and tea were recorded. Information on the addition of milk or cream to coffee and tea was not obtained since the major concern in the study was with "drugs" and no particular hypothesis related to beverages was entertained when the study was being designed.

For exposures to drugs and for consumption of coffee and tea the routine history taking concerned the three-month period before hospitalization. If the history was positive, further information was obtained. For coffee and tea, the use was quantitated in terms of 3 categories: none; intermediate (1 to 5 cups per day); and heavy (6 or more cups per day).

The patients were asked if they had ever had a "heart attack," "high blood pressure" or "diabetes."

Data acquisition was carried out by specially trained nurses. They conducted patient interviews within a few days of admission and extracted other information from patient charts. Subsequently, discharge diagnoses were obtained.

Subject Selection

The survey involved consecutive patients admitted to general medical and surgical wards.

Excluded from the survey were those who had been hospitalized during the three-month period immediately before the current hospitalization, were under 20 or over 75 years of age, or could not be interviewed (for medical reasons). In addition, an unknown fraction of patients who were hospitalized for less than 72 hours were missed because of time lags between patient admissions and interviews.

In the present analyses, some further general restrictions of inclusion were employed. The analyses were confined to white patients 40 to 69 years of age and to those without a discharge diagnosis of alcoholism. In addition, cases with a discharge diagnosis of acute myocardial infarction admitted to a surgical ward (9 in number) were excluded.

Cases. The identification of cases of myocardial infarction for the purposes of the present analyses was based on routine discharge diagnoses. This approach was adopted after assurance about reasonable accuracy of this diagnosis had been attained: for the first 406 patients hospitalized on the basis of acute chest pain with an admission diagnosis of possible or definite

The members of the Boston Collaborative Drug Surveillance Program, Boston University Medical Center, are as follows: co-directors, Drs. Hershel Jick and Dennis Slone; senior investigators, Drs. Samuel Shapiro, Olli P. Heinonen, Allen Mitchell, Chester Swett, Jr., and Micha Levy; biometry, Mr. Stuart C. Hartz; information science, Dr. Russell R. Miller; computer science, Mr. Leonard F. Gaetano and Dr. Dean S. MacLaughlin; and consultants in epidemiology and biometry, Drs. Olli S. Miettinen and Richard R. Monson (Harvard School of Public Health) and Martin P. Vessey (Oxford University). The following hospitals participated in this study: Beth Israel Hospital, Beverly Hospital, Boston University Hospital, Boston Veterans Administration Hospital, Brockton Hospital, Cardinal Cushing Hospital, Emerson Hospital, Faulkner Hospital, Framingham Union Hospital, Lawrence Memorial Hospital, Leonard Morse Hospital, Malden Hospital, Marlboro Hospital, Mt. Auburn Hospital, New England Medical Center, New England Memorial Hospital, Newton-Wellesley Hospital, Norwood Hospital, Quincy City Hospital, Salem Hospital, Sancta Maria Hospital, South Shore Hospital, Symmes Hospital and Waltham Hospital (address reprint requests to Dr. Jick at the Boston Collaborative Drug Surveillance Program, 400 Totten Pond Rd., Waltham, Mass. 02154).

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myocardial infarction, the records — apart from the discharge summary and diagnosis — were reviewed by an experienced cardiologist (Dr. H. E. Thomas). Using the WHO criteria,[7] he classified 225 of these cases as acute myocardial infarction whereas the remaining 181 did not meet the criteria. In these two groups, the frequencies of the discharge diagnosis were 197 (88 per cent) and 30 (17 per cent), respectively, so that, in this sample, 197 ÷ (197 + 30) = 87 per cent of those with a discharge diagnosis of acute myocardial infarction met the WHO criteria. This was considered adequate for the purposes of the study, and review of the remaining patients with the discharge diagnosis of acute myocardial infarction was not undertaken.

Control subjects. The possible need to restrict the control series in diagnostic terms was explored, since it was desirable to exclude patients whose condition is in general related to the use of coffee (either etiologically, via a confounding factor or through an influence by the disease on coffee habits). For the 12 most common diagnostic categories, the distribution according to level of coffee consumption was reviewed, standardizing for age and sex (Table 1). Patients with various diagnoses indicative of previously diagnosed coronary heart disease, such as "status post myocardial infarction" and "angina pectoris," drank somewhat less coffee than others (presumably because the disease leads to a reduction in coffee consumption). No material relation between any other diagnosis and coffee consumption was apparent. Of particular pertinence was a lack of association between coffee consumption and the diagnosis of diabetes, hypertension or obesity. On the basis of these observations, it was decided to include all patients without acute myocardial infarction in the control series, but to take a history of coronary-artery disease into account in the analysis.

Table 1. Distribution of Patients in the Most Common Diagnostic Categories According to Daily Consumption of Coffee.[a]

Diagnosis	No. of Patients	Percent Level of Patients Indicative Level of Coffee Consumption (Cups/Day)		
		0	1-5	6+
Acute MI	440	14.4	69.6	16.0
Status post MI	341	29.0	60.2	10.8
Cardiac dysrhythmia	225	38.7	50.7	10.6
Angina pectoris	158	34.3	55.4	10.7
Cerebrovascular disease	665	20.1	67.3	12.5
Hypertension	1,201	24.6	65.2	10.2
Diabetes	623	23.6	65.1	11.3
Cancer	780	23.1	67.3	9.6
Hemorrhoids	212	18.8	67.5	13.7
Arthritis	515	20.6	65.0	14.3
Diarrhea	608	22.0	65.6	12.4
Obesity	310	21.8	66.5	11.7
Other	6,864	22.6	64.3	13.1

[a] [illegible] & [illegible] patients [illegible] "other" [illegible] MI indicates [illegible]

[b] [illegible] heart disease, "coronary artery disease" & "congestive [illegible]

With these specifications, the present study was based on a total of 440 patients with the discharge diagnosis of acute myocardial infarction and a control series of 12,319 patients with other diagnoses.

Preliminary Analyses

There was no evidence of a positive association between tea drinking and acute myocardial infarction, the frequency of heavy tea drinkers among cases (7/440 = 1.6 per cent) being somewhat lower than among controls (234/12,319 = 1.9 per cent). By contrast the frequency of heavy coffee drinkers was higher among cases (15 per cent) than among controls (11 per cent) (Table 2).

Table 2. Distributions of Patients with Acute Myocardial Infarction (MI) and Controls According to Daily Consumption of Coffee.[a]

	No. of Subjects at Indicated Level of Coffee Consumption (Cups/Day)			
	0	1-5	6+	totals
Series:				
MI	71	302	67	440
Control	2,864	8,039	1,416	12,319
Estimate:				
CRR	(1.0)	1.5	1.9	
SRR[b]	(1.0)	1.6	2.2	
90% CLs for RR[c]		1.3, 2.0	1.6, 3.1	
p value (2-sided)		0.0006	<0.0001	

[a] Also shown are estimates of risk ratio (RR) both "crude" (CRR) & standardized (SRR) together with approximate 90% confidence limits (CL) & p values. Apart from the CRR, all computations took account of age, sex & history of MI (see text).

[b] These results were obtained with each of 2 standards: all controls & controls who do not drink coffee.

[c] Computations carried out under the assumption of uniformity of RR over the strata considering that the maximum likelihood estimates of presumed uniform RR are also 1.6 & 2.2 for those drinking 1-5 & 6 or more cups/day, respectively.

As a first step in the evaluation of the relation of coffee consumption to acute myocardial infarction, factors that confound the association (i.e., factors that, in the series under study, are associated with the use of coffee and which are, independently, related to the risk or ascertainment of acute myocardial infarction) were sought by the use of stratification and discriminant-function analysis. Both approaches confirmed the lack of association between acute myocardial infarction and the ingestion of tea. Nor was acute myocardial infarction associated with the addition of sugar to coffee: the frequencies of sugar use among coffee-drinking patients and controls were 55 and 60 per cent, respectively. Similarly, there was no confounding by occupation (professionals and managers, laborers, others) or hypertension (secondary discharge diagnosis), both being unrelated to coffee consumption. On the other hand, there was some confounding by each of the following factors: age, sex, presence of coronary heart disease (other than acute myocardial infarction), smoking, season, hospital and religion. However, most of these factors diluted rather than exaggerated the association between coffee consumption and acute myocardial infarction.

Final Analyses

In view of the complexity of the relation of both coffee consumption and acute infarction to a variety of factors (e.g., age, sex, past history of myocardial infarction) and to explore the association fully, the data were analyzed in three ways.

In the first place, the "crude" and standardized associations between acute myocardial infarction and coffee consumption were considered. The standardization involved age, sex and history of myocardial infarction.

Secondly, matched controls were selected for the cases. The matching factors were age (decade), sex, history of myocardial infarction, smoking (ex-smoker, current smoker, other), season (January to May, June to September) and hospital (each of the 24 hospitals). Up to two controls were sought for each case. With more than two potential control subjects available for a given patient with myocardial infarction, random sampling was employed.

Thirdly, as an additional means of controlling confounding, a multivariate score characterizing, for each subject, the individual risk for acute myocardial infarction was derived and employed. The score involved all the factors used in the matching, together with histories of the use of antianginal drugs (yes or no) and digitalis preparations (yes or no), diabetes (yes or no—based on discharge diagnosis for history of use of antidiabetic drug) and religion (Jewish or other). The score for each subject was taken to be the value of the linear discriminant function separating cases and controls. In addition to the specified factors, the function involved the level of coffee consumption. (In computing the score for the individual subjects, the level of coffee consumption for all subjects was set to zero.[4]) As expected, subjects with the lowest risk scores tended to be young, female, nonsmoking, etc., whereas those with the highest scores were particularly characterized by a history of coronary heart disease.

Results

The distributions of cases and controls according to level of coffee consumption are shown in Table 2. As compared to those who drink no coffee, the "crude" risk ratios of myocardial infarction among those who drink one to five and six or more cups of coffee daily are estimated to be 1.5 and 1.9, respectively.[4] With age, sex and history of myocardial infarction taken into account, the respective estimates of standardized risk ratio became 1.6 and 2.2.[4] According to the other results given in Table 2, one can be approximately 95 per cent confident that the respective risk ratios are at least 1.5 and 1.6, respectively,[5] and the two-sided p values from testing them against unity were 0.0006 and much less than 0.0001, respectively.

The risk contrast between the two extreme categories of coffee consumption was explored with closer control of confounding, first by use of matching on the six factors previously specified. Patients and controls drinking either zero or at least six cups of coffee daily were included. There were a total of 138 cases in these categories, and two matched controls could be found for 88 of them. The distribution of the matched triplets according to their patterns of coffee consumption is given in Table 3. The table shows that the numbers of patients drinking zero and at least six cups per day were 45 and 43, respectively, and that there were 20 matched triplets such that the patient and both the controls drank no coffee, etc. From these data, the two-sided p value is 0.02,[4] the point estimate of risk ratio is 1.9, and the approximate 95 per cent one-sided lower confidence limit is 1.2.[5] These data with two-to-one matching were augmented by data from 27 matched pairs representing cases in which there was only one available control patient. Of the 27 pairs, 13 were exposure-discordant, and eight of these favored the association under study. From this analysis, the two-sided p value is 0.005, the point estimate (maximum likelihood) of risk ratio is 2.0, and the approximate 95 per cent one-sided lower confidence limit of risk ratio is 1.3.

Table 3. Distribution of Matched Triplets Consisting of a Patient with Acute Myocardial Infarction (MI) and Two Controls According to Pattern of Coffee Consumption[*]

Daily No. of Cups of Coffee Consumption	No. of Controls Drinking 6 or More Cups/Day			Totals
	2	1	0	
6+	4	23	16	43
0	3	22	20	45

Estimate of risk ratio	1.9
90% confidence limits to risk ratio	1.2,3.0
p value (2-sided)	0.02

The data with stratification by the multivariate risk score are presented in Table 4. Again, patients and controls drinking either zero or at least six cups of coffee daily were evaluated. Within the range of the score that was common to both cases and controls there were 137 cases and 3533 controls. From these data the overall estimate of risk ratio is 2.2, with 90 per cent two-sided confidence limits of 1.6 and 3.0, and a two-sided p value well below 0.0001.

There is, in the data in Table 4, a suggestion that the strength of the association (risk ratio) is accentuated in those with high risk for myocardial infarction. However, this trend could be due to chance ($p < 0.08$). Repeating stratification by the same score for some subgroups yields the following risk-ratio estimates: for those with and without a history of myocardial infarction (55 and 82 cases), 3.1 and 1.8, respectively; for males and females (99 and 38 cases), 2.3 and 1.7, respectively; and for age categories 40 to 49, 50 to 59 and 60 to 69 years (30, 57 and 50 cases), 2.5, 1.9 and 2.5, respectively. All these variations are readily consistent with uniformity of the underlying risk ratio.

Table 4. Distributions of Patients with Acute Myocardial Infarction (MI) and Controls According to Level of Coffee Consumption.*

Risk Quintile† among Cases	Series	No. of Subjects at Indicated Level of Coffee Consumption (Cups/Day) 0	6+	Estimate of Risk Ratio
1	Cases	17	11	
	Controls	1,315	671	1.3
2	Cases	12	15	
	Controls	395	251	2.0
3	Cases	13	14	
	Controls	370	164	2.4
4	Cases	14	14	
	Controls	160	54	3.0
5	Cases	14	13	
	Controls	117	36	3.0
Estimate of risk ratio	2.2			
90% confidence limits for risk ratio	1.6,3.0			
p value (2-sided)	<0.0001			

*All subjects drinking <5 cups/day have been excluded. The subjects have been stratified according to a multivariate risk score in the range common to both cases & controls (see text).

†Cases of MI have been divided into 5 approximately equal strata. The 1st quintile represents the 20% of cases with the lowest risk scores, together with all control subjects in the range of that score. The 2d quintile represents 20% of cases with the next higher risk scores, together with all control subjects in the range of that score. A so on.

Discussion

The earlier finding of a positive association between coffee consumption and acute myocardial infarction[1] is corroborated by the present data. The statistical significance of the observed association is very high. Also, estimates of the magnitude of the association in the present study are similar to those obtained in our previous study: for those drinking at least six cups of coffee daily the risk is again estimated to be about twice that of nondrinkers.

The association of acute myocardial infarction with coffee consumption need not be regarded as inconsistent with the slightly negative associations of past myocardial infarction and angina pectoris with this habit. People with (overt) coronary heart disease may well tend to reduce their use of coffee. Whereas the data on past coffee habits were not available for direct evaluation of this possibility, indirect evidence of such a reduction may be read into the fact that ex-smokers were three times as common among those with past myocardial infarction as among those without such a history.

The observed association could be spurious rather than real. A spurious association would tend to arise under the following circumstances: if coffee consumption increased the likelihood of hospitalization for, or diagnosis of, acute myocardial infarction; if there were selective early mortality of patients drinking lesser amounts of coffee, or if acute myocardial infarction led to an exaggeration of the patient's level of coffee consumption, reflecting a bias on the part of the patient or the interviewer. The first and last possibilities can be readily excluded, because the data were collected before there was any general awareness about the association at issue, and because the nurse interviewers collecting the data were unaware of any special concern with myocardial infarction. As far as the second possibility is concerned, it should be noted that in acute myocardial infarction in general, the fatality rate before hospitalization is some 60 per cent,[10-12] and that the hospital fatality rate (among the remainder) is about 30 or 20 per cent depending on the availability of a coronary-care unit[13]; by contrast, in the present series only 4 per cent died during the hospitalization, and the failure to ascertain cases with an early fatal outcome is consistent with a spurious association through the second possibility.

Of potential confounding factors that might explain the association, the present study substantially eliminates the following: age, sex, pre-existing coronary heart disease, hypertension, congestive heart failure, obesity, diabetes, smoking and occupation. On the other hand, data on factors such as personality, diet and exercise were not available for analysis.

For a causal explanation it would be sufficient, in principle, to postulate that either sugar or milk (cream) as an additive to coffee is conducive to myocardial infarction.[14-16] In the present study, the data permitted a direct exclusion of the former possibility. Secondarily, this casts some doubt on an explanation in terms of milk as well, since the uses of these two additives are probably correlated. Moreover, milk is also used with tea, and, as in our previous study,[1] the use of tea showed a slight negative — though statistically quite nonsignificant — association with acute myocardial infarction.

Regarding the ingredients of coffee itself, an explanation of the association in terms of a pharmacologic effect of caffeine[17] must be viewed in the context of the apparent lack of association between tea consumption and acute myocardial infarction. The available information on the relative contents of caffeine in coffee and tea is somewhat wanting, but it appears to us that any effects of caffeine should be manifest among tea drinkers as well.[1]

As an alternative line of direct causal explanation, it has been suggested that ingredients other than caffeine in coffee inhibit fibrinogenolysis and fibrinolysis.[18]

Overall, it is our view that a causal interpretation of the association is not justifiable on the basis of currently available evidence. Moreover, the very existence of an association in healthy populations, such as those used in most epidemiologic cohort studies, cannot be inferred from our data. Nevertheless, in view of the ubiquitous use of coffee and the importance of myocardial infarction, the possibility that coffee contributes to the risk for myocardial infarction cannot be ignored.

We are indebted to the staffs and administrations of the participating hospitals for co-operation and especially to the record-room personnel for help.

References

1. Coffee drinking and acute myocardial infarction: report from the Boston Collaborative Drug Surveillance Program. Lancet 2:1278-1281, 1972
2. Ischaemic Heart Disease Registers: Report of the 5th Working Group, Copenhagen, World Health Organization, April 26-29, 1971
3. Miettinen OS, Neff RK: Computer processing of epidemiologic data. Hart Bull 2:98-103, 1971
4. Cornfield J: A method of estimating comparative rates from clinical data: applications to cancer of the lung, breast and cervix. J Natl Cancer Inst 11:1269-1275, 1951
5. Miettinen OS: Standardization of risk ratios. Am J Epidemiol 96:383-388, 1972
6. Gart JJ: Point and interval estimation of the common odds ratio in the combination of 2 × 2 tables with fixed marginals. Biometrika 57:471-475, 1970
7. Mantel N, Haenszel W: Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 22:719-748, 1959
8. Miettinen OS: Individual matching with multiple controls in the case of all-or-none responses. Biometrics 25:339-355, 1969
9. *Idem*: Estimation of relative risk from individually matched series. Biometrics 26:75-86, 1970
10. Armstrong A, Duncan B: Survey of acute coronary disease inside hospital. Br Heart J 32:555, 1970
11. Gordon T, Kannel WB: Premature mortality from coronary heart disease: the Framingham study. JAMA 21:1611-1621, 1971
12. Wikland B: Medically unattended fatal cases of ischaemic heart disease in a defined population. Acta Med Scand Suppl 524:1-78, 1971
13. Hofvendahl S: Influence of treatment in coronary care unit on prognosis in acute myocardial infarction: a controlled study of 771 cases. Acta Med Scand Suppl 519:9-78, 1971
14. Poston RN: Coffee drinking and acute myocardial infarction. Lancet 1:45-46, 1973
15. Segall JJ: Coffee drinking and acute myocardial infarction. Lancet 1:46, 1973
16. Dalderup LM: Coffee drinking and acute myocardial infarction. Lancet 1:104, 1973
17. Rajapakse DA: Coffee drinking and acute myocardial infarction. Lancet 1:313-314, 1973
18. Misirlioglu YI: Coffee drinking and acute myocardial infarction. Lancet 1:46, 1973

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Collaborative Study of a Method For The Determination of Caffeine in Nonalcoholic Beverages

By ARTHUR R. JOHNSON (Division of Food Standards and Additives, Food and Drug Administration, Washington, D.C. 20204)

A spectrochromatographic procedure for the determination of caffeine based upon modifications of the original Levine procedure was subjected to a collaborative study. Recoveries were generally better than 90%. The results indicate that the method is accurate for the determination of caffeine in nonalcoholic beverages. It is recommended that the method be adopted as official, first action.

In 1962, Levine (1) described a spectrochromatographic procedure for the determination of caffeine in drug tablets, beverages, and several coffee products. Several authors reported modifications of the basic column chromatographic procedure (2, 3). A method for the determination of caffeine in coffee products, reported by Borker and Sloman (4), was adopted as official, first action in 1964 (5).

With minor variations in sample preparation and column packing, this procedure was used for the determination of caffeine in nonalcoholic beverages and was studied collaboratively in 1965 with unsatisfactory results. It was recommended that further modifications be made and the study be continued the following year (6).

For the collaborative study of the revised method, samples were prepared from commercial cola-flavored sirup that had been decaffeinated. Five solutions, each consisting of 340 ml sirup, 10 g sodium benzoate, and sufficient distilled water to make 2 L of an acceptable 1 + 5 beverage mixture, were prepared to simulate beverage preparations. Caffeine was added to four of the solutions at levels of 5, 10, 20, and 40 mg/100 ml, respectively. One solution had no added caffeine and any absorbance found in this sample was attributed to the natural caffeine content of the sirup. The five samples were submitted to collaborators with instructions to analyze for caffeine by the method provided. A sample of caffeine was provided for standardization purposes.

METHOD

(Method is preferable to 8.037-8.039 when only few samples are to be analyzed. Use 8.032-8.036 for complex mixts requiring extensive cleanup.)

Apparatus

Chromatographic columns.—Glass, ca 25 cm × 250 mm.

Reagents

(Use $CHCl_3$ and ether washed with ½ vol. H_2O thruout.)

Caffeine standard solns.—(*1*) *Stock soln.*—100 mg caffeine/100 ml $CHCl_3$. (*2*) *Std solns.*—Prep. dilns of stock soln contg 0.25, 0.50, and 0.75 mg caffeine/50 ml $CHCl_3$. Check absorbance factors (*see Calculations*) frequently to minimize instrument variations.

Preparation of Sample

Remove any gas by pouring sample back and forth in beakers. Pipet appropriate amt sample (5 or 10 ml) into 250 ml beaker. Evap. to near dryness on steam bath. Dissolve residue in 5 ml NH_4OH (1 + 2) and heat 2 min. on steam bath. Remove from heat, add 6 g Celite 545, and mix carefully to homogeneous consistency before charging column.

Preparation of Columns

(a) *Column I.*—Carefully mix 2 ml 4*N* H_2SO_4 with 2 g Celite 545, place in chromatographic column over glass wool plug, and tamp to uniform mass with moderate pressure. Top with glass wool plug to minimize damage to column surface.

(b) *Column II.*—(*1*) *Layer A.*—Carefully mix 3 g Celite 545 with 2 ml 2*N* NaOH, transfer to column over glass wool plug, and tamp to uniform mass. (*2*) *Layer B.*—Transfer prepd sample and Celite mixt. to column directly over layer *A* and tamp. Dry wash

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beaker with 1–2 g dry Celite and transfer to column. Tamp to uniform mass and top column with glass wool plug.

Determination

Mount column II over column I. Pass 150 ml ether thru column II and into column I, using initial portion of ether to rinse sample beaker. Drain well and remove column II. Pass addnl 50 ml ether thru column I and drain well. Pass 50 ml $CHCl_3$ thru column I using initial portion to wash tip of column II. Collect eluate in 50 ml vol. flask and adjust vol. to 50 ml. Read absorbance of $CHCl_3$ soln at 276 mμ against $CHCl_3$ reference using Beckman DU with 1 cm silica cells, or other appropriate spectrophotometer. If necessary, adjust concns to acceptable levels by dilg with $CHCl_3$.

Calculations

Factor F = mg caffeine in 50 ml std/ absorbance reading.

mg Caffeine in sample = factor F × absorbance reading.

Express results as mg caffeine/100 ml beverage.

Results and Recommendation

The results of the study, including per cent recoveries, are summarized in Table 1. Recoveries were generally better than 90% and the method is considered to be acceptable.

It is recommended that the spectrochromatographic method, as described, be adopted as official, first action as an alternate method for the determination of caffeine in nonalcoholic beverages.

Table 1. Caffeine content (mg/100 ml) of collaborative samples

Coll.	Sample 1 (None Added)	Sample 2 (5 mg Added)	Sample 3 (10 mg Added)	Sample 4 (20 mg Added)	Sample 5 (40 mg Added)
1	0.0	4.7	9.2	19.0	37.0
2	0.3	5.5	9.8	19.8	39.4
3	0.0	4.6	9.2	19.0	35.2
4	0.0	4.8	9.2	18.7	38.1
5	0.0	5.4	9.4	18.2	37.1
6	0.1	5.0	9.9	19.1	38.0
7	0.0	5.0	9.5	17.7	35.6
8	0.0	5.7	9.9	20.5	37.5
9	0.4	5.2	9.2	16.7	37.4
Av.	0.09	5.10	9.48	18.75	37.26
% Recovery	—	102	95	94	93

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E. Fernandez-Flores and D. A. Kline, Food and Drug Administration, Washington, D.C.

The recommendation of the Associate Referee was approved by the General Referee and by Subcommittee D, and was adopted by the Association. See *This Journal* 50, 136 (1967).

References

(1) Levine, J., *This Journal* 45, 254–255 (1962).

(2) Yeransian, J. A., Kadin, H., Borker, E., and Stefanucci, A., *ibid.* 46, 315–319 (1963).

(3) Borker, E., *ibid.* 46, 319–320 (1963).

(4) Borker, E., and Sloman, Katherine G., *ibid.* 48, 705–709 (1965).

(5) "Changes in Methods," *ibid.* 48, 212 (1965).

(6) "Report of Subcommittee D," *ibid.* 48, 178–179 (1965).

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Caffeine Poisoning

By

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(Received February 21, 1959).

Caffeine, like the other methyl xanthines, is a relatively harmless substance. In small doses its effect is predominantly stimulating, and this accounts for the consumption of coffee, tea, etc., as stimulants and refreshments. Larger doses, in excess of about one gram, bring on a marked state of agitation, disturbances in heart function, pains of angina pectoris type, gastro-intestinal symptoms, acute diuresis, tremor of the muscles and even convulsions. People react in markedly different ways to caffeine, some can take considerable doses without any alarming symptoms, and this is probably most often the result of habitual use of coffee and other beverages containing the base.

Caffeine doses inducing toxic symptoms are 0.5–1 g (MEYER - GOTTLIEB 1936). The literature contains descriptions of cases of caffeine poisoning involving large doses of the drug, e. g., 20 g, of which admittedly part had been eliminated by vomiting and part by gastric lavage (SZIMZÓ 1934) and 4 g (ROUTH 1884), or immoderate quantities of coffee, as, for example, an extract prepared from 250 g of coffee beans (FORT 1885) and a decoction likewise prepared from 250 g coffee beans (LEWIN 1925), which probably corresponded to 3.75–5 g of caffeine.

No deaths caused by caffeine poisoning have been reported (PETRI 1930; KRANTZ & CARR 1958; MOLLER 1958). Certain textbooks of toxicology and pharmacology (FÜHNER 1951; DRILL 1958; MOESCHLIN 1958) mention 10 g as a lethal dose of caffeine; this corresponds to approximately 150 mg/kg. This presumably derives from tests carried out on laboratory animals (table 1).

Table 1.

(According to *Spector* 1956) Doses of caffeine lethal to test animals.

Animal	Route	Dose	Dosage mg/kg value
Frog	s. c.	LD	120–130
Mouse	s. c.	LD	180–190
	i. v.	LD_{50}	100.9 ± 6.67
Rat	oral	LD_{50}	233 ± 14
	s. c.	LD	70–250
	i. v.	LD_{50}	104.8 ± 1.87
Rabbit	oral	LD	290–360
	s. c.	LD	200–280
	i. v.	LD	80–100
Cat	oral	LD	100–150
	s. c.	LD	150–155
	i. v.	LD	80–100
Dog	oral	LD	140–150
	s. c.	LD	110
	i. v.	LD_{50}	175

Below is described a death from caffeine poisoning that occurred in a Helsinki mental hospital in 1951. The hospital had received from a pharmacy a delivery of sterile solutions of glucose (50 g/100 ml) and caffeine (Caffeina et Sodii Benzoas (former BP) = Coffeinum natrii benzoas (NFN)) 20 g/100 ml) in 100 ml bottles of the same size.

The hospital had never before received caffeine in such large bottles. It had been the practice in the drug storeroom of the hospital to label bottles according to the contents, degree of toxicity and to place them accordingly in different parts of the medicine cupboard. The pharmacist and the cleaner, who under the direction of the former attached the labels indicating the degree of toxicity of the contents, failed to notice among the glucose bottles the four bottles of caffeine, which were identical in size and packed in the same way, though provided with caffeine labels. By mistake D-labels, signifying harmlessness and intended for the glucose bottles, were also pasted on the caffeine bottles, which should have been provided with C-labels.

The mistake went undetected when a certain ward asked for glucose; among the glucose bottles delivered were two containing caffeine.

It was the practise in this ward to place several bottles of glucose, syringes and hypodermic needles on a tray in preparation for injections to revive patients in a state of insulin shock. The tray was then taken into the patients' rooms.

When on this occasion the turn of a 35-year-old woman patient came to be revived, the duty nurse in training filled a 20 ml syringe from one of the bottles on the tray without looking at the original label and handed the syringe to the nurse in charge of the ward, who also did not inspect the bottle from which the pupil had taken the solution, but injected the liquid

into a vein in the patient's arm. The patient responded with a violent fit of convulsions, but the nurse assumed that insulin was the cause and asked for more glucose from the student nurse. The latter again filled the syringe from the same bottle and the patient received 20 ml more of the same solution. The patient again underwent convulsions, grew pale, as if dying, stopped breathing and, in spite of artificial respiration, died shortly after.

The nurse in charge and the student nurse, however, went on reviving the insulin-treated patients and gave the next patient also an intravenous injection from the same bottle. After about 5 ml of this caffeine solution, thought to contain glucose, had been injected into a vein in the patient's arm, the patient had a violent convulsion and looked bad. The nurse then immediately stopped the injection and, on inspecting the bottle from which the solution had been taken, noticed that it contained caffeine. After receiving glucose without delay, the patient revived in the usual way.

The bottle in question thus contained 0.2 g caffeine and sodium benzoate/1 ml, or 0.08 g caffeine/1 ml. The patient who died had received intravenously about 40 ml, which contain approximately 3.2 g of caffeine, corresponding to about 57 mg/kg.

The *autopsy* revealed the immediate cause of death as a sudden state of violent shock. It should be borne in mind, however, that the patient had been in a state of insulin shock upon receiving the caffeine injections. However, since the patient had undergone insulin shock several times before and had reacted normally to it, and since death from insulin shock is relatively rare (1/200), caffeine poisoning may be considered the primary cause of death. The course of this caffeine poisoning corresponds closely with observations on laboratory animals, in which even small intravenous doses of caffeine induce convulsions, a steep drop in blood pressure, disturbances in heart function and finally diastolic arrest.

It is noteworthy that the intravenous dose of 5 ml (of the same caffeine solution) given the other patient sufficed to bring on a violent convulsion. Five ml of the caffeine solution contained 0.4 g of pure caffeine.

Summary.

The authors describe a case of death from caffeine poisoning. A patient in insulin shock was intravenously given a caffeine solution by mistake, instead of glucose, the amount of the drug injected being 3.2 g. Another patient received an intravenous injection of 0.4 g caffeine and underwent a brief fit of convulsions.

REFERENCES

Drill, V. A.: *Pharmacology in Medicine*. 2. Ed. New York-Toronto-London 1958, 305.

Fort: *Journ. de Medicine* 1885. Cited by Lewin 1929.

Lühner, H.: *Medizinische Toxikologie*. 3. Aufl. Stuttgart 1951, 185.

Gloganer: *Therap. Mhefte* 1888, 2, 197.

Krantz, J. C. & C. J. Carr: *The Pharmacologic Principles of Medical Practice*. 4. Ed. Baltimore 1958, 664.

Lewin, L.: *Die Fruchtabtreibung durch Gifte und andere Mittel*. 4. Aufl. Berlin 1921, 427.

Lewin, L.: *Gifte und Vergiftungen*. 4. Aufl. Berlin 1929, 747.

Meyer, H. H. & R. Gottlieb: *Die experimentelle Pharmakologie*. 9. Aufl. Wien 1936, 397.

Moeschlin, S.: *Klinik und Therapie der Vergiftungen*. 3. Aufl. Stuttgart 1958, 426.

Moller, K.: *Pharmakologie*. 3. Aufl. Basel 1958, 523.

Muller: *Therap. Mhefte* 1890, 4, 314.

Petri, Else: *Pathologische Anatomie und Histologie der Vergiftungen*. Berlin 1930, 364.

Routh, C. H.: *Lancet* 1883, 680.

Spector, W. S.: *Handbook of Toxicology*. Philadelphia & London 1956, Vol. I, 56.

Szemzo, G.: *Wien. klin. Wschr.* 1934, 47, 560.

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COFFEE AND CORONARY DISEASE

CURRENT assessment of the epidemiology of coronary heart disease indicates a major role of environment in the evolution of this disease. Environmental factors that contribute to the incidence of the disease include dietary habits, cigarette smoking and physical activity. The present issue of the *Journal* contains an article incriminating coffee drinking as a factor in the development of coronary heart disease. The report of Jick et al. involves a study of coffee consumption by a large number of hospitalized subjects with a discharge diagnosis of myocardial infarction as compared with subjects hospitalized for other reasons. The findings indicate a higher coffee intake among the patients with myocardial infarction before the episode leading to hospitalization. From these findings the authors estimated that coffee drinkers were at a twofold increased risk of development of infarction.

The report, "Coffee and Myocardial Infarction," confirms a previous one[1] but is in disagreement with data from prospective studies. Curiously enough, coffee is associated with myocardial infarction, but tea is not. As with almost all retrospective studies the subjects investigated constituted only those with myocardial infarction who survived long enough to be hospitalized. This group of patients experienced a mortality rate of only 4 per cent. Such a sample is hardly respresentative of this highly lethal disease.

The Achilles heel of any retrospective study is the control group against which the subjects with disease are to be compared. Jick et al. used discharged hospitalized patients. Such a population may contain an excessive number of subjects with gastrointestinal or other diseases in which coffee drinking has been either voluntarily abandoned or medically proscribed. Thus, the coffee consumption of a hospitalized population cannot be accepted unequivocally as representative of the habits of the general population.

Although retrospective studies are of proved value in the development of hypotheses, for more definitive studies they seldom provide conclusive evidence regarding real differences between those in whom a disease develops and those remaining free of it. To test the hypothesis, it is usually necessary to observe the development of the disease in a general population. Such studies avoid any distortion in selection of either disease or control groups.

The relation of coffee intake to coronary heart disease in general and myocardial infarction in particular has already been investigated in a number of prospective studies such as Framingham, Chicago and elsewhere.[2] The prospective studies of which we are aware either have failed to find any association between coffee intake and myocardial infarction or have observed an associated characteristic that could account for the relation. In these prospective studies no relation of antecedent coffee intake to subsequent myocardial infarction, regardless of outcome, has been observed.

How consistent is the evidence linking coffee to the

occurrence of myocardial infarction in retrospective studies? Some have found no difference,[1] and others have found an excessive intake of tea and less of coffee in subjects with myocardial infarction.[1]

The pharmacologic basis that suggested a possible relation between coffee or tea and the development of coronary heart disease was the xanthine content of these beverages. Both coffee and tea contain caffeine, and tea contains theophylline. The finding of Jick and his associates that tea is innocuous and coffee harmful to the cardiovascular system is paradoxical. This observation led these authors to blame some other unknown ingredient in coffee. Other investigators have suggested the harmful effects of sucrose or cream in the coffee — or associated cigarette smoking.[2]

Even if we accept caffeine intake as a possible mechanism by which coffee exerts an adverse influence on the cardiovascular system we find that studies of the acute effect of caffeine on the cardiovascular system have shown only minor increases in blood pressure and heart rate. Dilatation of the coronary vessels is counterbalanced by the increased demand imposed by slight stimulation of the myocardium. The chronic effects of coffee intake are at present unknown, but tolerance certainly develops. Contradictory effects on atherogenesis have been reported.

It is a common lament that almost everything that one enjoys in life is either illegal, immoral or fattening. To these we must add — and hazardous to the heart. Must we now forgo the traditional coffee break and morning cup of coffee? The retrospective data presented by Jick et al. suggest that we should.

However, careful examination of all the evidence would counsel against drawing premature conclusions. Jick and his associates apparently concur in this, for they show laudable restraint in interpreting their data. Paul, who provided the first prospective data seeming to incriminate coffee, has also advised caution. In fact, in multivariate analysis his data suggest that cigarette smokers who drink coffee are better off.[6] Clearly, more evidence is needed before we can confidently add coffee to the growing list of faulty living habits that must be curtailed in the quest for a lower coronary incidence. We are faced with a formidable enough task in having to engineer physical activity back into daily living, change cigarette-smoking habits and modify the national diet. We should not complicate the problem further by advocating premature restrictions of other pleasurable habits unless there is equally good evidence that such a change is warranted.

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REFERENCES

1. Coffee drinking and acute myocardial infarction: report from the Boston Collaborative Drug Surveillance Program. Lancet 2:1278-1281, 1972
2. Paul O, MacMillan A, McKean H, et al: Sucrose intake and coronary-heart disease. Lancet 2:1049-1051, 1968
3. United States Department of Health, Education and Welfare, National Heart Institute: The Framingham Heart Study: Habits and coronary heart disease (PHS Publication No 1545) Washington DC: Government Printing Office 1966
4. Walker WJ, Gregoratos MG: Myocardial infarction in young men. Am J Cardiol 19:339-344, 1967
5. Brown A: Coronary thrombosis: an environmental study. Br Med J 2:567-573, 1962
6. Paul O: Stimulants and coronaries. Postgrad Med 44(5):196-199, 1968

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The Effect of Daily Ingestion of Caffeine on the Microsomal Enzymes of Rat Liver*

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Abstract—The effect of repeated ingestion of caffeine on rat liver microsomal enzymes was studied. In the rat, ingestion of 30-24 mg caffeine kg/day in the drinking water for more than 2 wk inhibited aminopyrine *N*-demethylase activity. Doses of activity approached 60% at the end of an 8-wk dosing schedule. Acetanilide hydroxylating activity of rat liver microsomal enzyme was initially stimulated, with a peak (145—[illegible]% of control) at 2 wk, and returned to normal levels at the end of 8 wk. Caffeine-treated rats did not respond to the phenobarbitone stimulation of microsomal enzymes to the same extent as did rats treated with phenobarbitone alone.

INTRODUCTION

In addition to the caffeine in coffee, tea and other beverages, caffeine is present in many commercial preparations consumed by the general public (Gleason, Gosselin, Hodge & Smith, 1969), so that substantial amounts of caffeine are ingested by large numbers of people. Caffeine, a xanthine alkaloid, has several pharmacological and biochemical effects, including stimulation of the central nervous system, diuresis (Fruitt, 1965), mutagenesis (Ostertag, Duisberg & Stürmann, 1965), inhibition of phosphodiesterase (Beavo, Rogers, Crofford, Baird, Hardman, Sutherland & Newman, 1971) and inhibition of DNA polymerase (Wragg, Carr & Ross, 1967). Psychotropic effects and tolerance to caffeine have also been reported (Colton, Gosselin & Smith, 1968; Goldstein, Kaizer & Whitby, 1969).

Caffeine is readily absorbed following ingestion and is distributed in the body according to the water content of the tissues (Bertoli, Dragoni & Podari, 1968). It is metabolized in mammals by demethylation to methylxanthines and to methyluric acids by oxidation at the C-8 position (Cornish & Christman, 1957; Khanna, Rao & Cornish, 1972; Parke, 1968).

Acute doses of caffeine have been shown to stimulate hepatic, drug-metabolizing microsomal enzymes in the rat (Mitoma, Sorich & Neubauer, 1968). It has been suggested (Lombrozo & Mitoma, 1970) that the effect of caffeine on cytochrome P_{450} is similar to that of 3-methylcholanthrene. Other than a single reference (Cornish, Wilson & Abdi, 1970), reporting that in rats treated with caffeine for 6 wk *N*-dealkylation activity was 66% of that in controls, no information was evident in the literature concerning the effect of repeated ingestion of caffeine on drug metabolizing microsomal enzymes. Since caffeine is consumed by the public in large quantities, the present study on the effect of prolonged ingestion of caffeine on microsomal enzymes was undertaken.

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EXPERIMENTAL

Animals and maintenance. Male rats of the Sprague–Dawley strain, weighing 250–300 g, were used for these studies. They were fed Rockland Rat Chow *ad lib.* and housed in stainless-steel cages.

Conduct of experiments. Caffeine was administered in the drinking-water (0·2 mg/ml), the daily dose being calculated on the basis of a daily consumption of 25–30 ml water/rat. In the first experiment, groups of rats (six treated animals and two controls) were killed at intervals between 4 and 56 days from the start of caffeine administration for hepatic microsomal-enzyme determinations. In the second study, caffeine was administered for 6 wk, on the last 3 days of which sodium phenobarbitone (25 mg/kg/day) was injected ip into some of the treated and control animals. Enzyme induction of the liver microsomal enzymes was determined by measurement of acetanilide-hydroxylation and aminopyrine-*N*-demethylation activities. Values in the caffeine/phenobarbitone-treated rats were compared with those in rats treated only with caffeine or only with phenobarbitone and with those in untreated rats of the same age. In the third study, caffeine was administered for 11 wk, and phenobarbitone (25 mg/kg) was injected ip on the last 3 days of treatment. Animals were killed 24 hr after the last dose for the determination of hepatic microsomal levels of cytochrome P_{450} and cytochrome b_5.

Fractionation of liver samples. The rats were anaesthetized with ether and killed by open-chest surgery. They were bled by heart puncture with the aid of vacutainers. Liver samples (5 g) were immediately frozen on dry ice and stored at −20°C before use. Each 5 g sample was homogenized in 10 ml 0·05 M-tris buffer (pH 7·4) containing 1·15% KCl by means of glass homogenizers and a teflon pestle fitted to a mechanical drill press. The homogenate was centrifuged at 13,000 g in a Sorvall refrigerated high-speed centrifuge. This supernatant was used for microsomal-enzyme analysis.

Enzyme and other assays. The incubation mixture (total volume 5 ml) contained supernatant (1 ml), substrates (17·8 μM acetanilide for hydroxylation and 3·5 μM aminopyrine for demethylation) and the following co-factors: glucose 6-phosphate (25 μM), NADP (2·25 μM), nicotinamide (20 μM), NAD (2·25 μM) and magnesium sulphate (17 μM) in 4 ml 0·1 M phosphate buffer (pH 7·4). This mixture was incubated under oxygen for 20 min in 25 ml Erlenmeyer flasks using a Dubnoff shaking incubator at 37°C. For *N*-demethylation studies, aminopyrine was used as a substrate and 4-aminoantipyrine was measured as the reaction product by the method outlined by Brodie & Axelrod (1950). Hydroxylation activity was determined essentially by the method of Brodie & Axelrod (1948), using acetanilide as a substrate. The enzyme activity was expressed as μg product formed/g liver/20 min. Cytochrome P_{450} and cytochrome b_5 values were determined by the method of Omura & Sato (1964) and expressed as nmoles/mg protein. A Beckman DK-2A Spectrophotometer was used for absorbancy measurements. Protein concentrations were measured as described by Lowry, Rosebrough, Farr & Randall (1951). The Student's *t* test was used for statistical evaluation of the results.

RESULTS

It was established that the average increase (233 ± 24 g) in the body weight of rats given caffeine (20–24 mg/kg/day) orally for a period of 12 wk was essentially the same as that of controls (202 ± 49 g). The liver weight/100 g body weight (3·58 ± 0·11 g) of rats fed caffeine was also comparable with that of untreated rats (3·67 ± 0·12 g).

Table 1 shows the effect of daily caffeine ingestion (20–24 mg/kg) on the mixed-function drug-metabolizing microsomal enzymes of the livers of rats killed at intervals of 4–56 days

Table 1. *Hepatic microsomal-enzyme activity in rats given 20–24 mg caffeine/kg/day in the drinking-water*

Duration of dosing (days)	Acetanilide hydroxylation (% of control)*	Aminopyrine N-demethylation (% of control)*
4	123 ± 4	115 ± 7
8	127 ± 12	104 ± 11
14	145 ± 11	90 ± 6
28	132 ± 7	73 ± 3
56	93 ± 9	38 ± 5

*Values are means for treated rats at each sacrifice period ± SEM. Two control rats were killed at each time.

from the start of treatment. Acetanilide hydroxylation and aminopyrine N-demethylation activities of these enzymes appeared to be minimally stimulated after a period of 4 days to approximately the same level (hydroxylation 123 ± 4% and N-demethylation 115 ± 7% of controls). The hydroxylation activity of these enzymes increased gradually with time, having a peak (145 ± 11% of control) at 2 wk and returning essentially to control level at 8 wk (93 ± 9%). In contrast, the mean activity of the N-demethylating enzyme showed only a slight initial elevation, with values declining over the subsequent study period. A marked decrease in N-demethylation activity was observed after 4 wk (73 ± 3% of control; $P < 0.05$). This loss of activity progressed as treatment continued, the N-demethylation enzyme levels of caffeine-treated animals being only 38 ± 5% of that of controls at the end of the 8-wk period.

In the second study, the phenobarbitone-treated animals showed the highest levels of acetanilide-hydroxylating activity (200 ± 13%) and aminopyrine N-demethylating activity (186 ± 19%), whereas animals treated with caffeine plus phenobarbitone had enzyme activities of 157 ± 12 and 140 ± 9% respectively (Table 2). This suggests that caffeine-treated animals were not able to respond to the phenobarbitone treatment to the same extent as did animals treated only with phenobarbitone.

Table 2. *Effect of phenobarbitone on the hepatic microsomal enzymes of rats given 20–24 mg caffeine/kg/day in the drinking-water for 6 wk*

Treatment	No. of animals/group	Acetanilide hydroxylation (% of control)*	Aminopyrine N-demethylation (% of control)*
None	3	100 ± 8	100 ± 5
Caffeine	6	110 ± 12	80 ± 11
Phenobarbitone†	5	200 ± 13	186 ± 19
Phenobarbitone† + caffeine	6	157 ± 12	140 ± 9

*Values are means for the numbers of animals shown ± SEM.
†Phenobarbitone (25 mg/kg) was injected ip on each of the last 3 days of the caffeine treatment.

Cytochrome P_{450} and cytochrome b_5 values of rats fed caffeine for 14 wk are given in Table 3. Rats dosed orally with caffeine (20–24 mg/kg) daily for 14 wk showed no change

Table 3. *Effect of phenobarbitone administration on cytochrome P_{450} and b_5 levels of hepatic microsomes of rats given 20–24 mg caffeine/kg/day for 14 wk*

Treatment	No. of animals/group	Cytochrome P_{450} (nmoles/mg protein)*	Cytochrome b_5 (nmoles/mg protein)*
None	4	1·00 ± 0·09	1·00 ± 0·[illegible]
Caffeine	6	1·13 ± 0·05	0·98 ± 0·06
Caffeine + phenobarbitone†	6	1·60 ± 0·11	0·71 ± 0·05

*Values are means for the numbers of animals shown ± SEM.
†Phenobarbitone (25 mg/kg) was injected ip on each of the last 3 days of caffeine treatment. The animals were sacrificed 24 hr after the last dose.

in their mean cytochrome P_{450} or cytochrome b_5 values when compared with the controls. However, caffeine-treated rats subsequently administered sodium phenobarbitone (25 mg/kg, ip) for the last 3 days of the treatment showed an increase in P_{450} level in comparison with both controls and caffeine-dosed animals. Cytochrome b_5 levels were essentially the same in caffeine-treated animals and in controls. In contrast to cytochrome P_{450} values, however, cytochrome b_5 levels were decreased in rats given both caffeine and phenobarbitone. The decrease was statistically significant ($P < 0{\cdot}01$) when compared with caffeine treated rats.

DISCUSSION

The prolonged ingestion of 20–24 mg caffeine/kg/day by normal, healthy rats does not seem to affect their growth rate. However, alterations in environmental temperature and the amount and quality of the diet have been reported to affect the toxicity of caffeine. Muller & Vernikos-Danellis (1970) showed that a slight increase or decrease in the normal environmental temperature (22 ± 7°C) of mice enhanced the toxicity of caffeine, and when food intake was less than half the voluntary intake, an increase in the toxicity of caffeine was noted (Peters, 1966).

The effect of short-term exposure to caffeine on the drug-metabolizing liver microsomal enzymes has been previously reported (Mitoma *et al.* 1958; Mitoma, Bombrozo, Le Valley & Dehn, 1969). Mitoma *et al.* (1969) reported that caffeine, tea and coffee in acute doses induced the *O*-demethylation of *O*-nitroanisole and the hydroxylation of acetanilide by rat-liver microsomes. Similarly, the present study shows some initial elevations of the microsomal-enzyme activity. However, our results (Table 1) show that the prolonged ingestion of caffeine by the rat markedly reduced the aminopyrine *N*-demethylation activity of rat liver. In contrast to *N*-demethylation, acetanilide-hydroxylating activity of the liver, although initially stimulated, was nearly normal after 8 wk of caffeine ingestion.

The mechanism of the stimulation of the drug-metabolizing enzymes of rat-liver microsomes by acute dosing with caffeine has not been satisfactorily elucidated. Stimulation of these microsomal enzymes by caffeine was shown to be partially inhibited by actinomycin D (Mitoma *et al.* 1969). Proof of enzyme induction, as measured by the increase in the incorporation of labelled amino acids into the microsomal proteins, was inconclusive. This

suggests that caffeine may stimulate microsomal-enzyme activity without causing an increase in enzyme synthesis. Caffeine and phenobarbitone in acute doses were shown by Mitoma *et al.* (1968) to have an additive effect with respect to the stimulation of the hydroxylation of acetanilide by the microsomal enzymes. On the other hand, this effect was not additive when *O*-nitroanisole or aminopyrine was used as the substrate. In the present [illegible] phenobarbitone stimulated hepatic microsomal enzmyes in rats that had been fed caffeine for 5 wk. This stimulatory effect of phenobarbitone on both the hydroxylation of acetanilide and the *N*-demethylation of aminopyrine was not additive (Table 2). In fact, it appears that, on prolonged ingestion, caffeine hinders the induction of microsomal enzymes by phenobarbitone.

Cytochrome P_{450} and the cytochrome b_5 contents of rat hepatic microsomes were evaluated in order to study the effects of caffeine on the electron transport system of mixed-function oxygenases. Cytochrome P_{450} levels in the liver microsomes of rats dosed with caffeine were essentially the same as those in the untreated rats. Similar results on cytochrome P_{450} contents of hepatic microsomes from rats given caffeine in acute doses had been reported by Lombrozo & Mitoma (1970). However, these workers suggested a possible alteration in the nature of the cytochrome P_{450} of liver microsomes from caffeine-treated rats. Recently, cytochrome b_5 of hepatic microsomes has been shown to be involved in the hepatic drug-metabolizing microsomal-enzyme system (Estabrook, Franklin, Cohen, Shigamatzo & Hildebrandt, 1971). Cytochrome b_5 is postulated as being responsible for the transfer of an electron to cytochrome P_{450} during the metabolism of substrates. It is rate-limiting only when the NADPH levels are low in the reaction mixture. Interestingly enough, cytochrome b_5 values are shown (Table 3) to be significantly decreased in the rats given caffeine and phenobarbitone. Studies in our laboratories (K. L. Khanna & H. H. Cornish, unpublished data, 1971) have shown that rats treated with phenobarbitone alone have increased levels of cytochrome P_{450} but not of cytochrome b_5. The mechanism by which caffeine and phenobarbitone-treated rats inhibit the synthesis or estimation of cytochrome b_5 is not clear.

In conclusion, rats fed caffeine for more than 2 wk begin to show a diminution of aminopyrine-*N*-demethylating activity in the liver. This loss of activity approaches 60% at the end of an 8-wk dosing schedule. Acetanilide-hydroxylating activity was not similarly affected. Although phenobarbitone stimulated microsomal-enzyme activity in caffeine-treated animals, the extent of induction was considerably below that found in control animals receiving only phenobarbitone. In addition, phenobarbitone stimulated production of cytochrome P_{450} in caffeine-treated animals, but cytochrome b_5 levels in the same animals were considerably below normal.

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REFERENCES

Beavo, J. A., Rogers, N. L., Crofford, O. B., Baird, E. E., Hardman, J. G., Sutherland, E. W. & Newman, E. V. (1971). Effects of phosphodiesterase inhibitors on cyclic AMP levels and on lipolysis. *Ann. N.Y. Acad. Sci.* **185**, 129.

Bertoli, M. A., Dragoni, G. & Rodari, A. (1968). Tissue distribution of labeled caffeine in mice. *Med. Nucl. Radiobiol. Lat.* **11**, 231.

Brodie, B. B. & Axelrod, J. (1948). The estimation of acetanilide and its metabolic products, aniline, N-acetyl p-aminophenol and p-aminophenol (free and total conjugated) in biological fluids and tissues. *J. Pharmacol. exp. Ther.* **94**, 22.

Brodie, B. B. & Axelrod, J. (1950). The fate of aminopyrine (Pyramidon) in man and methods for the estimation of aminopyrine and its metabolites in biological materials. *J. Pharmacol. exp. Ther.* 99, 171.
Colton, T., Gosselin, R. E. & Smith, R. P. (1968). The tolerance of coffee drinkers to caffeine. *Clin. Pharmac. Ther.* 9, 31.
Cornish, H. H. & Christman, A. A. (1957). A study of the metabolism of theobromine, theophylline, and caffeine in man. *J. biol. Chem.* 228, 315.
Cornish, H. H., Wilson, Carol E. & Abar, Ellen L. (1970). Effect of foreign compounds on liver microsomal enzymes. *Am. ind. Hyg. Ass. J.* 31, 605.
Estabrook, R. W., Franklin, M. R., Cohen, B., Shigematzu, A. & Hildebrandt, A. G. (1971). Influence of hepatic microsomal mixed function oxidation reactions on cellular metabolic control. *Metabolism* 20, 187.
Gleason, Marion N., Gosselin, R. E., Hodge, H. C. & Smith, R. P. (1969). *Clinical Toxicology of Commercial Products*. 3rd ed. Section V, p. 108. The Williams & Wilkins Co., Baltimore.
Goldstein, A., Kaizer, Sophia & Whitby, O. (1969). Psychotropic effects of caffeine in man. IV. Quantitative and qualitative differences associated with habituation to coffee. *Clin. Pharmac. Ther.* 10, 489.
Khanna, K. L., Rao, G. S. & Cornish, H. H. (1972). Metabolism of caffeine-^{14}H in the rat. Abstracts: Eleventh Annual Meeting, Society of Toxicology, Williamsburg, Va. *Toxic. appl. Pharmac.* 22, 292.
Lombrozo, L. & Mitoma, C. (1970). Effect of caffeine on hepatic microsomal cytochrome P-450. *Biochem. Pharmac.* 19, 2317.
Lowry, O. H., Rosebrough, Nira J., Farr, A. L. & Randall, Rose J. (1951). Protein measurement with the Folin phenol reagent. *J. biol. Chem.* 193, 265.
Mitoma, C., Lombrozo, L., LeValley, Susanna E. & Dehn, Frances (1969). Nature of the effect of caffeine on the drug-metabolizing enzymes. *Archs Biochem. Biophys.* 134, 434.
Mitoma, C., Sotsch, H. T. J. & Neubauer, Susanne F. (1968). The effect of caffeine on drug metabolism. *Life Sci.* 7, 145.
Muller, Patricia J. & Vernikos-Danellis, Joan (1970). Effect of environmental temperature on the toxicity of caffeine and dextroamphetamine in mice. *J. Pharmac. exp. Ther.* 174, 152.
Omura, T. & Sato, R. (1964). The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J. biol. Chem.* 239, 2370.
Ostertag, W., Duisberg, E. & Stürmann, M. (1965). The mutagenic activity of caffeine in man. *Mutation Res.* 2, 293.
Parke, D. V. (1968). *The Biochemistry of Foreign Compounds*. p. 152. Pergamon Press, Ltd., London.
Peters, J. M. (1966). Caffeine toxicity in starved rats. *Toxic. appl. Pharmac.* 9, 390.
Truitt, E. B., Jr. (1965). In *Drill's Pharmacology in Medicine*. 3rd ed. Edited by J. R. DiPalma. p. 394. McGraw-Hill Book Company, New York.
Wragg, June B., Carr, Jacqueline V. & Ross, Virginia C. (1967). Inhibition of DNA polymerase activity by caffeine in a mammalian cell line. *J. Cell Biol.* 35, 146A.

Effet de l'ingestion quotidienne de caféine sur les enzymes microsomiques du foie chez le rat

Résumé—On a étudié chez le rat l'effet de l'ingestion répétée de caféine sur les enzymes microsomiques du foie. L'ingestion quotidienne, pendant plus de 2 semaines, de 20–24 mg/kg de caféine ajoutée à l'eau de boisson a inhibé l'activité de l'aminopyrine-N-déméthylase. Cette diminution d'activité s'est élevée à près de 60% à la fin d'un traitement de 8 semaines. L'activité d'hydroxylation de l'acétanilide de l'enzyme microsomique du foie du rat était d'abord stimulée, atteignant un maximum (145 ± 11% par rapport aux animaux témoins) à 2 semaines et revenait à des niveaux normaux à la fin des 8 semaines. Les rats traités à la caféine ne réagissaient pas au même degré à la stimulation par le phénobarbital des enzymes microsomiques que ceux traités uniquement au phénobarbital.

Der Einfluss täglicher Ingestion von Koffein auf die mikrosomalen Enzyme der Rattenleber

Zusammenfassung—Der Einfluss wiederholter Ingestion von Koffein auf mikrosomale Enzyme der Rattenleber wurde untersucht. Bei der Ratte hemmte die Aufnahme von 20–24 mg Koffein/kg/Tag im Trinkwasser für die Dauer von über 2 Wochen die Aktivität von Aminopyrin-*N*-demethylase. Dieser Aktivitätsverlust kam gegen das Ende einer 8-Wochen-Verabreichungsperiode 60% nahe. Die Acetanilidhydroxylierungsaktivität des mikrosomalen Rattenleberenzyms wurde anfänglich angeregt, wobei nach 2 Wochen ein Maximum (145 ± 11% der Kontrolle) erreicht wurde, und kehrte nach 8 Wochen zu normalen Werten zurück. Koffeinbehandelte Ratten reagierten nicht auf die Phenobarbitone-Stimulierung von mikrosomalen Enzymen im gleichen Ausmass wie Ratten, die nur mit Phenobarbitone allein behandelt worden waren.

FATAL POISONING BY CAFFEINE (A CASE FROM PRACTICE)

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Caffeine is distinguished by its relatively low toxicity. However even with the application of 0.2-0.5 g, there is onset of certain symptoms of poisoning, and excessive doses (greater than 1 g of pure caffeine) evoke the appearance of great agitation, accompanied by hallucination, confused thought, vertigo, fever, tremor in the extremities, convulsions, extreme headache, increased pulse and respiration, vomiting, diarrhea, and decline in heart activity. Usually even in severe cases, a complete recovery occurs in a period of 1-2 days. A case is described in which, a5 minutes after a dose of 4 g of caffeine, the patient exhibited a burning sensation in the chest, tremors, vertigo, diarrhea, severe diuresis, pain in the abdomen, a very rapid pulse, and collapse. After nine hours the major symptoms of poisoning leveled off, and on the third day, the patient was practically healthy again. Chronic poisoning by caffeine is possible under industrial conditions by breathingtea dust, as well as in tasters who ingest samples of strong tea and coffee. Poisoning also occurs in the case of excessive consumption of strong tea. Acute poisoning is not noted under industrial conditions.

M. P. Nikolayev and other authers indicate the possibility of acute poisoning in people by caffeine with fatal outcome, with the application of about 10 g of caffeine. People suffering from heart disease are especially sensitive to caffeine. Experiments with animals have established that a fatal dose of 1 kg of caffeine is equal to 0.2 g for horses, 0.3 g for cows, goats, and swine, and 0.5 g for dogs. Death results from heart stoppage.

In practical laboratory studies, a case of fatal poisoning caffeine occurred in a woman 47 years old. It was established that the deceased suffered, when alive, from shortness of breath, she was not treated in a medical facility, and she often took small doses of caffeine. On the day of death, she took by mistake approximately 250 ml of a 20% caffeine-sodium benzoate solution, i. e. approximately 19 g of pure caffeine, after which vomiting and diarrhea occurred. Death occurred about 30 minutes after ingestion. No characteristic anatomical changes in internal organs were found when the body was discovered.

A judicial investigation revealed 0.265 g of caffeine in 200 g weight of the internal organs.

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СМЕРТЕЛЬНОЕ ОТРАВЛЕНИЕ КОФЕИНОМ

(Случай из практики)

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Республиканское бюро (нач. А. В. Шпиганович) судебномедицинской экспертизы Министерства здравоохранения Латвийской ССР

Кофеин отличается сравнительно малой ядовитостью, однако при приеме 0,2—0,5 г наступают уже некоторые симптомы отравления, а чрезмерные дозы (свыше 1 г чистого кофеина) вызывают явления сильного возбуждения, сопровождающиеся галлюцинациями, спутанностью мыслей, головокружением, лихорадкой, дрожанием конечностей, судорогами, сильной головной болью, учащением пульса и дыхания, рвотой, поносом и упадком сердечной деятельности. Обычно даже в тяжелых случаях полное выздоровление наступает в течение 1—2 суток. Описан случай, когда по принятии 4 г кофеина спустя 15 минут у больного появилось чувство жжения в груди, дрожь, головокружение, понос, сильный диурез, боли в животе, очень частый пульс и коллапс. Спустя 9 часов основные симптомы отравления сгладились, а на 3-й день больной практически был здоров. Хронические отравления кофеином возможны в производственных условиях при вдыхании чайной пыли, а также у дегустаторов при взятии пробы крепкого чая или кофе. Встречаются также отравления в быту при чрезмерном потреблении крепкого чая. Острых отравлений в производственных условиях не отмечено.

М. П. Николаев и другие авторы указывают на возможность острого отравления людей кофеином со смертельным исходом при приеме около 10 г кофеина. Особенно чувствительны к кофеину лица, страдающие сердечными заболеваниями. Опытами на животных установлено, что смертельная доза кофеина на 1 кг веса для лошадей равна 0,2 г, для коров, коз, свиней — 0,3 г, для собак — 0,5 г. Смерть наступает от остановки сердца.

В практике работы лаборатории имел место случай смертельного отравления кофеином женщины, 47 лет. Установлено, что покойная при жизни страдала одышкой, в лечебные учреждения не обращалась, часто принимала небольшие дозы кофеина. В день смерти ошибочно приняла около 250 мл 20% раствора кофеин-бензоата натрия, т. е. около 19 г чистого кофеина, после чего наблюдались рвота и понос. Смерть наступила примерно через 30 минут после приема. Каких-либо характерных анатомических изменений внутренних органов при вскрытии трупа не обнаружено.

При судебнохимическом исследовании в 200 г внутренних органов найдено 0,265 г кофеина.

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Coffee Drinking Prior to Acute Myocardial Infarction

Results From the Kaiser-Permanente Epidemiologic Study of Myocardial Infarction

Arthur L. Klatsky, MD; Gary D. Friedman, MD, MS; Abraham B. Siegelaub, MS

No independent association between coffee drinking and a subsequent first myocardial infarction in 464 patients was found in a study well controlled for cigarette smoking and five other established "risk factors." Coffee drinking and cigarette smoking are strongly correlated habits. Review of other studies of coffee and coronary heart disease indicates that discrepancies in findings may be related either to cigarette smoking or to selection of control subjects. Coffee drinking is not an established risk factor for myocardial infarction.

A recent report of possible association between coffee drinking and acute myocardial infarction[1] received widespread publicity in the lay press and stimulated a lively correspondence in the medical press.[2-13] Earlier epidemiologic investigations had either failed to show such a correlation[14] or had disclosed a moderately positive association[15] that the same authors later[16] attributed largely to association of coffee drinking and cigarette-smoking habits. In a search for predictors of myocardial infarction additional to those generally recognized, we have examined a wide range of variables recorded during multiphasic health checkups (MHCs) (the Kaiser-Permanente Epidemiologic Study of Myocardial Infarction; the study design in detail and results for standard risk factors will be published elsewhere[17]). The data from that study were carefully controlled for six predictors of myocardial infarction ("risk factors"), including cigarette smoking. These data show no association between coffee drinking and a subsequent first myocardial infarction.

Subjects and Methods

The subjects were 464 persons (88% [408/464] white) who had undergone an MHC in the years 1964 to 1970. The MHC questionnaire included a single question relating to coffee drinking ("Have you usually, in the past year, drunk more than six cups of coffee per day?"). Each of these subjects subsequently suffered a first myocardial infarction. The diagnosis of myocardial infarction was documented by careful review of the hospital chart, with rigidly applied clinical, electrocardiographic, and serum enzymatic criteria. Ninety-four patients had a prior diagnosis of some manifestation of coronary heart disease, such as angina pectoris, but patients were excluded if review indicated any evidence of myocardial infarction previous to the MHC.

The positive or negative responses of these persons to the question about coffee drinking were compared to the answers of controls who had not suffered a myocardial infarction. Computer search of 250,000 MHC records from the same years yielded, for each patient with myocardial infarction, two controls: an "ordinary" and a "risk" control. The ordinary control was matched for age, sex, skin color, and MHC date. The risk control was closely matched for these factors, except for MHC date, and in addition, for the presence or absence of an electrocardiographic diagnosis of "no significant abnormality" and for current cigarette smoking; this control was also matched (wherever possible, within the same population quintile) for systolic and diastolic blood pressure, serum cholesterol level, and serum glucose level with the patient with myocardial infarction. The success of the matching process made

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Table 1.—Results of Risk Factor Matching

Trait	Myocardial Infarction Cases N	Myocardial Infarction Cases Mean	Risk Controls N	Risk Controls Mean
Age, yr	464	58.9	464	59.0
Cholesterol, mg/100 ml	425	253.4	464	253.4
Systolic blood pressure, mm Hg	440	151.9	463	149.9
Diastolic blood pressure, mm Hg	440	85.6	463	85.3
Glucose, mg/100 ml 1 hr after 75-gm oral load	459	190.9	464	191.9
Current cigarette smoking	433	46.7%	462	47.2%
Electrocardiographic abnormality, any type	460	44.6%	463	43.5%

Table 2.—Percentages of Myocardial Infarction Patients and Controls Who Reported Drinking More Than Six Cups of Coffee Daily

Age, yr	% Drinking >6 Cups Daily: MI Patients	Ordinary Controls	Risk Controls	No. of Pairs* MI Patients vs Risk Controls
Total	21.4	18.9	22.3	457
>65	9.4	13.6	10.7	148
55-64	23.7	17.7	25.3	152
<55	30.4	25.0	30.2	157
Men	22.6	20.6	22.9	328
>65	8.2	12.2	11.1	98
55-64	25.0	20.2	22.7	108
<55	21.1	27.6	32.6	122
Women	18.3	14.6	20.8	129
>65	11.8	16.3	10.0	50
55-64	20.5	11.4†	31.8†	44
<55	25.0	16.2	22.2	35
Prior clinical diagnosis of coronary heart disease other than MI				
Yes	18.1	11.7	19.2	94
No	22.2	20.8	23.1	363

*In each subgroup and total group, the number of pairs of myocardial infarction (MI) patients vs ordinary controls and of ordinary controls vs risk controls was within two of the number of pairs of MI patients vs risk controls.
†$P<.05$.

possible by the large number of available records is demonstrated in Table 1.

Results

With respect to the frequency of positive responses to the question about consumption of more than six cups of coffee daily, the only statistically significant difference among patient and control groups appeared in the small subgroup of women between 55 and 64 years of age (Table 2), where more risk controls than ordinary controls gave affirmative answers ($P<.05$). In no subgroup did the critical comparison, patients with myocardial infarction vs risk controls, show a statistically significant difference.

About 20% of all patients with myocardial infarction, of all ordinary controls, and of all risk controls said that they drank more than six cups of coffee daily. In each of these three groups, the proportion of coffee drinkers decreased sharply in the last two decades studied. These findings may be compared with data compiled on 111,000 *unselected* MHC examinees (Table 3). The overall percentages of coffee drinkers in that large sample were 19.5% for women and 21.4% for men. The highest proportions of persons who answered the question affirmatively (20% to 25%) appeared in the three decades 30 to 59 years, the proportions diminishing sharply thereafter. These findings are strikingly similar to those in our three study groups.

Previous studies of this subject have attempted to deal with the problem presented by a strong association between coffee-drinking and cigarette-smoking habits. The strength of this association in a large sample of the population taking MHCs is seen in the fact that only 9.7% of white male nonsmokers reported drinking more than six cups of coffee per day, whereas 33.8% of white male current cigarette smokers stated that they drank a like amount of coffee. Corresponding figures for white women were 12.8% of nonsmokers vs 30.9% of smokers in the more than six cups of coffee daily group.

As might be expected in our three study groups, there was a large excess of current cigarette smokers in the myocardial infarction and risk-control groups (both 47%) compared to the ordinary control group (31%; $P<.001$). (Myocardial infarction cases and risk-control groups were matched for smoking.) The slightly lower proportion of coffee drinkers (not statistically significant) in the ordinary control group compared to the other two groups probably reflects an association with cigarette smoking.

As a test of the relationship of coffee drinking and cigarette-smoking habits in the myocardial infarction subjects and in the two control groups, subgroups of matched pairs of cases and risk controls were examined further to provide even closer matching on smoking habits. Refined definitions developed in an earlier study of MHC examinees[15] were used. By these definitions, "nonsmokers" were individuals who had never smoked, currently or in the past. Of 74 such matched pairs, 10.8% of the subjects and 10.8% of the risk controls reported that they drank more than six cups of coffee per day. "Current established cigarette smokers" were defined as those who stated that they smoked cigarettes both during the past year and previously. Of 166 such matched pairs, 28.9% of the patients with myocardial infarction and 36.8% of the risk controls answered the question affirmatively. Comparisons of these strictly defined case and risk-control groups with corresponding ordinary controls also failed to show statistically significant differences in the proportions of persons reporting

daily consumption of more than six cups of coffee.

Comment

It is beyond the scope of this brief report to discuss in detail the statistical problems involved in the study of a possible factor or trait in relationship to myocardial infarction or other manifestations of coronary heart disease. It is widely accepted that multiple factors are known to be related to these conditions and that there are additional factors not yet uncovered. When a factor is found to be correlated with coronary heart disease independently of already known risk factors, it remains possible that the association might be to some additional unknown risk factor or trait. Traits likely to be associated with coffee-drinking habits are other personal habits, national origin, occupation, personality, and climate of residence. Association with these or other traits could account for discrepancies among findings in various studies.

In the Framingham Heart Study,[14] a weakly positive but statistically insignificant correlation was found between drinking more than five cups of coffee per day and the subsequent development of clinical coronary heart disease. The published data were apparently not controlled for smoking. The authors concluded that "coronary heart disease was not affected by moderate amounts of coffee." The Western Electric Study[15,16] showed only a weak relationship of coffee drinking to later coronary heart disease independent of tobacco usage. The Kaiser-Permanente Epidemiologic Study of Myocardial Infarction was specifically designed to demonstrate, without multivariate analysis, traits predictive of myocardial infarction independent of the risk factors for which matching was done. In these three studies with essentially negative findings concerning coffee drinking and myocardial infarction, the data about coffee drinking were recorded prior to the coronary events under study. These studies also used as controls ambulatory persons who were essentially well, or at least who had presumably no more illness than the populations from which they were drawn.

In contrast to these three studies, the Boston Collaborative Drug Study[1] utilized matched controls derived from patients hospitalized at the same institutions as the myocardial infarction cases. These controls included 10% with cancer, 15% with chronic respiratory disease, 11% with hypertension, and 14% with diabetes. The information about coffee drinking and tea consumption in myocardial infarction cases and in controls was collected during hospitalization for their illnesses. There was an unusually small difference in proportions of cigarette smokers between the myocardial infarction and control groups. The authors suggested that the controls may have had a high proportion of smokers because many had tobacco-associated diseases. Since the process of control selection may also have affected the proportion of coffee users in any study, a tabulation of the percentage of coffee drinkers in various studies is instructive (Table 4).

The Boston investigators[1] found low percentages of coffee drinkers in the non-US hospitals, presumably inversely related to high tea consumption in these countries. (Tea consumption was found not to be related to myocardial infarction.) In the hospitals in the Boston area, 20% of the studied patients with myocardial infarction drank large amounts of coffee—a proportion closely similar to that observed by us in patients with infarction, in our control groups, and in our unselected MHC population. It is also similar to available percentages of heavy coffee drinkers in the other studies. The proportion of heavy coffee drinkers among controls in the Boston area hospitals (10%) was far lower than in any other study. It is possible that this low percentage reflected avoidance of coffee by seriously, chronically ill, hospitalized patients. This attempt at explanation does not, however, explain why the number of tea consumers was not also less among these controls who were ill. It is conceivable that persons who might avoid coffee would drink tea under the widespread belief that tea is a milder and less stimulating beverage.

The possibility that coffee drinking is a substantially less prevalent habit in New England than in other regions of the US must also be considered. Data showing coffee consumption for the years 1963 to 1972 in various regions of the US[19] is shown in Table 5.

Table 3.—Percentages of 111,000 Unselected MHC Examinees Who Reported Drinking More Than Six Cups of Coffee Daily

	Total, All Ages	Age, yr					
		20-29	30-39	40-49	50-59	60-69	70-79
Men	21.4	16.1	24.4	25.6	23.8	15.6	8.7
Women	19.5	11.9	23.5	24.9	22.1	14.8	10.1

Table 4.—Percent of High-Intake Coffee Drinkers in Coronary Heart Disease (CHD) Studies

Study, Source	Amount of Coffee in High-Intake Categories	Patients in High-Intake Categories, % (No.)
Western Electric Co, Paul et al[15,16]	>150 cups/mo (5+ cups/day)	22 Non-CHD (244/1,108) 41 CHD* (22/54)
National Research Council (male veteran twins),† Hrubec[18]	6+ cups/day	24 (2,575/10,744)
Kaiser-Permanente Epidemiologic Study, this report	>6 cups/day	21 MI (98/464) 22 Risk controls (103/464) 19 Ordinary controls (87/464)
Boston Collaborative Drug Surveillance Program[1]‡		
Total group	6+ cups/day	15 MI (40/276) 9 Controls (100/1,104)
US hospitals		20 MI (27/136) 10 Controls (56/544)
Non-US hospitals		9 MI (13/140) 8 Controls (44/560)

*Later shown that excess in coronary heart disease (CHD) largely related to associated cigarette smoking.
†Data concern coffee drinking and cigarette smoking in relationship to angina pectoris. Consistent independent relationship of coffee drinking to angina not found.
‡This study was based on data from eight hospitals; four in the Boston area and four non-US hospitals.

Table 5.—Coffee Consumption in the US, 1963-1972*

Year	Region: East	Midwest	South	West
1963	2.76	3.30	2.54	3.56
1964	2.54	3.20	2.61	3.38
1965	2.50	3.14	2.48	3.09
1966	2.66	3.23	2.53	3.00
1967	2.63	3.18	2.39	3.19
1968	2.49	3.03	2.40	3.02
1969	2.43	2.92	2.42	3.03
1970	2.30	2.83	2.31	2.92
1971	2.36	2.86	2.02	2.77
1972	2.10	2.66	1.97	2.74

*Statistics based on cups per person per day. Study includes all persons 10 years of age and older.

Interestingly, there was a consistent, slightly lower average per capita consumption of coffee in the eastern part of the US than in the west through these years. The magnitude of the regional difference was approximately 20%. Unfortunately, the source does not supply data about consumers of large amounts of coffee, and it does not supply information about the Boston and San Francisco areas specifically. It seems unlikely to us that the small regional difference in coffee-drinking habits provides the major explanation for the discrepancy under consideration.

Others have speculated about specific components of coffee (caffeine, sucrose, and cream) in possible relationship to coronary heart disease. It seems inappropriate for us to comment on this since we find no overall relationship to coffee drinking, and since we believe that the preponderance of evidence does not support an association. With regard to any clinical inferences to be derived from this subject, it certainly seems premature to add to the privations already inflicted on the actual or potential coronary disease victim (Spartan diet, abstinence from tobacco, avoidance of slothful habits) by denying him the solace of his morning coffee.

Subsequent to submission of this report, a second study from the Boston Collaborative Drug Surveillance Program has been reported by Jick et al (*New Engl J Med* 289 [pt 2]:63-67, 1973). The study confirms the association reported earlier with a similar procedure for selection of control subjects. Among those who consumed large amounts of coffee, the ratio of the myocardial infarction patients to the control patients was 15.2% (67/440) to 11.5% (1,416/12,319), respectively.

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Dr. Collen was largely responsible for the MHC program and the statistical data base that we used; Dr. Crawford directed the multiphasic testing program in San Francisco; and Dr. Feldman was the Multiphasic Coordinator in Oakland, Calif.

References

1. Coffee drinking and acute myocardial infarction, Boston Collaborative Drug Surveillance Program. *Lancet* 2:1278-1281, 1978.

2. Poston RN: Coffee drinking and acute myocardial infarction. *Lancet* 1:45-46, 1973.

3. Segall JJ: Coffee drinking and acute myocardial infarction. *Lancet* 1:46, 1973.

4. Dalderup LM: Coffee drinking and acute myocardial infarction. *Lancet* 1:104, 1973.

5. Misirlioglu YI: Coffee drinking and acute myocardial infarction. *Lancet* 1:46, 1973.

6. Nichols AB: Coffee drinking and acute myocardial infarction. *Lancet* 1:480-481, 1973.

7. Steinke J: Coffee drinking and acute myocardial infarction. *Lancet* 1:258, 1973.

8. Hayreh SS: Coffee drinking and acute myocardial infarction. *Lancet* 1:45, 1973.

9. Rajapakse DA: Coffee drinking and acute myocardial infarction. *Lancet* 1:313-314, 1973.

10. Hrubec Z: Coffee drinking and ischemic heart disease. *Lancet* 1:548, 1973.

11. Bradshaw S: Coffee drinking and ischemic heart disease. *Lancet* 1:548-549, 1973.

12. Bignall JC: Coffee drinking and ischemic heart disease. *Lancet* 1:548, 1973.

13. Seakins JWT: Coffee drinking and ischemic heart disease. *Lancet* 1:666, 1973.

14. *The Framingham Heart Study: Habits and Coronary Heart Disease.* Public Health Service publication 1515. US Government Printing Office, 1966.

15. Paul O, et al: A longitudinal study of coronary heart disease. *Circulation* 28:20-36, 1963.

16. Paul O, et al: Sucrose intake and coronary heart disease. *Lancet* 2:1049-1051, 1968.

17. Friedman GD, et al: Kaiser-Permanente epidemiologic study of myocardial infarction: Study design and results for standard risk factors. *Am J Epidemiol*, to be published.

18. Friedman GD, et al: Smoking among white, black and yellow men and women. *Am J Epidemiol* 96:23-35, 1972.

19. *Coffee Drinking in the United States.* New York, Pan-American Coffee Bureau, 1972, p 14.

Farm. Zh. 24(4):52-53. 1969.

THE PHOTOELECTROCOLORIMETRIC METHOD FOR DETERMINING CAFFEINE

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In the quantitative determination of caffeine, an unknown quantity can be described by the colorimetric (4-6) and spectrophotometric methods (2,7). Recently, Arzamastseva (1) improved the conditions of the colorimetric determination of caffeine and 8-methyl caffeine, using the color reaction of products from the alkaline dissociation of caffeine, by means of the diazo salts "dark violet K". She showed that the products of the alkaline dissociation of caffeine give a color with phosphomolybdic acid.

We set out to use the above-mentioned reaction for the photoelectrocolorimetric determination of caffeine in solutions and in some medicinal forms. The results indicated that the most reproductive values of optical density of the coloring solutions which we obtained in this case, with dissociation of caffeine in an alkaline medium, were produced at room temperature + or - 10 deg F. The most important factor, which is the cause of the intensity of the color, is the amount in milliliters of phosphomolbdic acid and its concentration. The most stable values of optical density were obtained for additions of 5 ml of freshly prepared 10% solution of phosphomolybdic acid in 3% nitric acid.

The technique of determining caffeine by this method is as follows. To 2 ml of solution made from 0.1 to 3 mg caffeine-sodium benzoate or from 0.05 to 1.5 mg of caffeine base add 2 ml of 35% solution of caustic soda and keep within 10 deg F of room temperature. To the mixture add 2 ml of 50% solution of phosphomolybdic acid, mix, and add 2 ml of 10% solution of phosphomolybdic acid and 4 ml of water. The optical density is measured with the aid of the FEC-M photoelectrocolorimeter (with a red filter, and a 10.05 mm diameter cuvette). For the amounts of caffeine in the experimental samples, a calibrating curve was drawn as an aid. To draw the curve, caffeine or caffeine-sodium benzoate is taken as a base, which corresponds to the requirements of the Derzhav Pharmacopoeia of the USSR (7) and which is made as outlined above. The fresh preparations of the color solutions of caffeine-sodium benzoate are depedent of the Lambert-Beer law in the concentration range 0.1-3 mg, for a caffeine base - in the range of 0.05-1.5 mg in the samples. The method is sensitive for 0.1 mg caffeine-sodium benzoate in a 15-ml solution and for 0.05 mg caffeine base in 15 ml solution.

TABLE. RESULTS OF QUANTITATIVE DETERMINATION OF CAFFEINE-SODIUM BENZOATE AND CAFFEINE BASE IN AQUEOUS SOLUTIONS AND IN MEDICINAL FORMS

Composition of Medicinal Form	Taken in Analysis ml	Taken in Analysis mg	Detected in mg	Detected in %	Results of Statistical Analysis $\bar{X}$	σ	$\sigma_{\bar{X}}$	t_p	A
0.20 caffeine-Na benzoate 0.25 NaBr 200.0 water	2.0 2.0 2.0	2.0 2.0 2.0	2.01 1.98 1.99	100.5 99.0 99.5	1.993	0.016	0.009	0.039	±1.95
10% caffeine-Na benzoate, 1.0 in ampoules	2.0 2.0 2.0	2.0 2.0 2.0	2.02 2.00 1.96	101.0 100.0 98.0	2.000	0.030	0.012	0.051	±2.55
0.25 caffeine-Na benzoate 200.0 water	2.0 2.0 2.0	2.5 2.5 2.5	2.48 2.52 2.50	99.20 100.80 100.00	2.500	0.020	0.012	0.051	±2.04
0.10 caffeine base, 200.0 water	2.0 2.0 2.0	1.0 1.0 1.0	0.99 1.00 0.98	99.0 100.0 98.0	[illegible]	0.010	0.006	0.025	±2.50

This method was used to determine caffeine-sodium benzoate and caffeine base in aqueous solutions, as well as in certain medicinal forms (solutions, ampoules). For the analysis specially prepared mixtures were taken of exactly known amounts of caffeine. The data obtained were statistically reliable.

In analyzing the solutions of caffeine-sodium benzoate and sodium bromide, we determined caffeine directly in solution, for 2 ml of the experimental solution.

In order to carry out the analysis of the ampoules, 1 ml of the solution was transferred in proportional amount to 100 ml and brought up to 100 ml with water. For the quantitative determination , 2 ml of the solution obtained was used. The results of the investigations, presented in table, show that the photoelectrocolorimetric method de cribed above permits the determination of caffeine base and caffeine-sodium benzoate with sufficient accuracy in certain medicinal forms. The presence of such components as amidopyrine, analgene, or codeine, complicate the determination because these substances give a color in phopshomolybdic acid.

CONCLUSIONS

1. The conditions were studied for the photoelectrocolorimetric determination of caffeine and caffeine-sodium benzoate. The method is based on the dissociation of caffeine in an alkaline medium and on the presence of a reaction with phosphomolybdic acid.

2. The method indicated permits the determination of caffeine base and caffeine-sodium benzoate in certain medicinal forms with an accuracy of up to + or - 2.5%.

LITERATURE CITED

1. Arzamastseva, A. P. (1958), Aptech. Delo, Vol. 7, No. 5, p. 68.
2. Kiripenko, L. O. (1966), Farmatsev. Zhur., No. 4, p. 43.
3. State Pharmacopoeia of the USSR (.961), 9th ed., Medgiz, Moscow, p. 126.
4.

Deniges G. C., R. hebd. seances. Acad. Sci., 1934, 199, 1022.—5. Nieh-ler I. I., Zebensmittell Untersuch. u. Forsch. r., 1942, 83, 107.—6. Danust. J. Am. Pharm. Assoc., Sci. Ed., 1953, 42, 747.—7. Devi I. G., Kherana M. L., J. Pharm., 1953, 10, 15.

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ФОТОЕЛЕКТРОКОЛОРИМЕТРИЧНИЙ МЕТОД ВИЗНАЧЕННЯ КОФЕЇНУ

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Для кількісного визначення кофеїну описана незначна кількість колориметричних (4—6) та спектрофотометричних методів (2, 7). Останнім часом Арзамасцев (1) розробив умови колориметричного визначення кофеїну та 8-метилкофеїну, використавши кольорову реакцію продуктів лужного розкладу кофеїну з солями діазонію «темно-фіолетовим К». Він показав, що продукти лужного розкладу кофеїну дають забарвлення з фосфорномолібденовою кислотою.

Ми поставили собі за мету використати вищезгадану реакцію для фотоелектроколориметричного визначення кофеїну в розчинах та деяких лікарських формах. Досліди показали, що найбільш репродуктивні значення оптичної густини забарвлених розчинів ми одержували в тому випадку, коли розклад кофеїну у присутності лугу проводили при кімнатній температурі на протязі 10 *хв*. Важливим фактором, який впливає на інтенсивність забарвлення, є кількість мілілітрів фосфорномолібденової кислоти та її концентрація. Найбільш постійні значення оптичної густини були одержані при додаванні 5 *мл* свіжовиготовленого 10% розчину фосфорномолібденової кислоти в 3% азотній кислоті.

Техніка визначення кофеїну за цим методом така: до 2 *мл* розчину, який вміщує від 0,1 до 3 *мг* кофеїну-бензоату натрію або від 0,05 до 1,5 *мг* основи кофеїну, додають 2 *мл* 35% розчину їдкого натру і залишають на 10 *хв* при кімнатній температурі. До суміші додають 2 *мл* 50% розчину оцтової кислоти, перемішують, додають 5 *мл* 10% розчину фосфорномолібденової кислоти і 4 *мл* води. Оптичну густину вимірюють за допомогою фотоелектроколориметра ФЕК-М (світлофільтр червоний, кювета з товщиною шару рідини в 10,05 *мм*). Вміст кофеїну в досліджуваних пробах визначають за допомогою калібрувальної кривої. Для побудови калібрувальної кривої беруть основу кофеїну або кофеїн-бензоат натрію, які відповідають вимогам Державної фармакопеї СРСР (7) і роблять, як вказано вище. Світлопоглинання забарвлених розчинів кофеїну-бензоату натрію підлягає закону Ламберта—

Результати кількісного визначення кофеїну-бензоату натрію та основи кофеїну у водних розчинах і в лікарських формах

Склад лікарської форми	Взято на аналіз в		Знайдено в		Результати статистичної обробки				
	мл	*мг*	*мг*	%	$\bar{x}$	σ	$^{\sigma}\bar{x}$	t_p	Δ
Кофеїну-бензоату натрію 0,20	2,0	2,0	2,01	100,5					
Натрію броміду 0,25	2,0	2,0	1,98	99,0	1,993	0,016	0,009	0,039	±1,96
Води 200,0	2,0	2,0	1,99	99,5					
Кофеїну-бензоату натрію 10% 1,0 в ампулах	2,0	2,0	2,02	101,0					
	2,0	2,0	2,00	100,0	2,000	0,020	0,012	0,051	±2,55
	2,0	2,0	1,96	99,0					
Кофеїну-бензоату натрію 0,25	2,0	2,5	2,48	98,20					
	2,0	2,5	2,52	100,80	2,500	0,020	0,012	0,051	±2,04
Води 200,0	2,0	2,5	2,50	100,00					
Кофеїну основи 0,10	2,0	1,0	0,99	99,0					
Води 200,0	2,0	1,0	1,00	100,0	0,99	0,010	0,006	0,025	±2,60
	2,0	1,0	0,98	98,0					

Бера в межах концентрацій 0,1—3 *мг*, для основи кофеїну — в межах 0,05—1,5 *мг* в пробі. Чутливість методу для кофеїну-бензоату натрію 0,1 *мг* в 15 *мл* розчину, для основи кофеїну — 0,05 *мг* в 15 *мл* розчину.

Цей метод був використаний нами для визначення кофеїну-бензоату натрію та основи кофеїну у водних розчинах, а також в деяких лікарських формах (розчини, ампули). Для аналізу були взяті спеціально виготовлені суміші з точно відомим вмістом кофеїну. Одержані дані були статистично оброблені.

При аналізі розчину, який вміщує кофеїн-бензоат натрію та натрію бромід, ми визначали кофеїн безпосередньо в розчині, взявши для нього 2 *мл* досліджуваного розчину.

Щоб провести аналіз ампул, 1 *мл* розчину кількісно переносили в мірну колбу на 100 *мл* і доводили водою до 100 *мл*. Для кількісного визначення використовували 2 *мл* одержаного розчину. Результати дослідів, наведені в таблиці, свідчать про те, що вищеописаний фотоелектроколориметричний метод дозволяє визначати кофеїну основу та кофеїну-бензоат натрію з достатньою точністю в деяких лікарських формах. Присутність таких компонентів, як амідопірин, анальгін, кодеїн, утруднює визначення, бо ці речовини дають забарвлення з фосфорномолібденовою кислотою.

ВИСНОВКИ

1. Вивчено умови фотоелектроколориметричного визначення кофеїну та кофеїну-бензоату натрію. Метод базується на розкладі кофеїну в лужному середовищі і наступній реакції з фосфорномолібденовою кислотою.

2. Вказаний метод дозволяє визначати основу кофеїну та кофеїн-бензоат натрію в деяких лікарських формах з точністю до ± 2,5%.

ЛІТЕРАТУРА

1. Арзамасцева А. П., Аптечное дело, 1958, 7, № 5, 68.— 2. Кириченко Л. О., Фармацевтичний журнал, 1966, № 4, 43.— 3. Государственная фармакопея СССР, IX изд., М., Медгиз, 1961, 126.

4. Deniges G. C., R. hebd. seances, Acad. Sci., 1934, 199, 1622.— 5. Richter I. I., Zebensmittell Untersuch. u. Fortsch. r., 1943, 98, 107.—6. Daoust, J. Am. Pharm. Assoc., Sci. Ed., 1953, 42, 747.— 7. Devl I. G., Khorana M. L., J. Pharm., 1953, 10, 15.

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PHOTOELECTROCOLORIMETRIC METHOD OF DETERMINATION OF CAFFEINE

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SUMMARY

The conditions have been studied of photoelectrocolorimetric determination of caffeine sodium benzoate and caffeine. The method is based on dissociation in alkaline medium and subsequent reaction with phosphomolybdic acid. The abovementioned method enables to determine caffeine in some medicinal forms with precision up to 2.5%.

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Extreme Sensitivity of Obese Hyperglycemic Mice to Caffeine and Coffee

By Dubravko M. Kuftinec and Jean Mayer

Mice with the hereditary obese hyperglycemic syndrome are unusually sensitive to the toxic action of caffeine. A single injection of caffeine, or the replacement of drinking water by coffee for 1 week causes a drastic and prolonged (2 or more months) elevation of blood glucose. Administration of purines and pyrimidines other than alloxan (shown previously to cause in this syndrome prolonged lowering of blood glucose and regranulation of beta cells) did not lead to striking results. No explanation for this unexpected effect of caffeine is offered.

THE HEREDITARY RECESSIVE HYPERGLYCEMIC SYNDROME in mice is characterized inter alia by extreme adiposity, moderate hyperphagia but extreme inactivity, a high blood glucose despite high pancreatic and circulating insulin-like activity, hypercholesterolemia, extreme sensitivity to cold, and insulin resistance.[1] The adipose tissue of animals with this syndrome shows enormously increased capacity for lipogenesis from acetate despite decreased glucose uptake.[2] Fat mobilization in these animals is not increased by epinephrine or F.M.S.[3] The syndrome may be due to the fact that the adipose tissue of these animals contains glycerokinase.[4] Because these animals show a paradoxic response to alloxan[3] with blood glucose decreased and appearance of massive granulation in beta cells of the islets of Langerhans, we decided to investigate the action of substances related chemically to alloxan—purines, pyrimidines and one alkaloid—caffeine. The effect of caffeine was so striking that it seems important to report it, particularly in view of our inability to find any study of the effect of this substance, or of coffee, in human beings with obesity and maturity onset diabetes, a condition which in many ways resembles the hereditary hyperglycemic syndrome.

Results

Toxicity

Obese hyperglycemic mice were found to be much more sensitive to the toxic effects of caffeine than normal animals. The L.D. 50 was determined in 42 obese and 28 nonobese animals. Under conditions of ad libitum feeding it was found to be 10 mg./animal for the lean animals (400 mg./Kg. of body weight) and 3.5 mg. for obese hyperglycemic animals (80 mg./Kg. of body

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Table 1.—*Effect of Caffeine on Blood Glucose Levels in Obese Hyperglycemic Mice (OH) and Lean Littermates (L)*

Hours	0	3	6	9	12	24	48
OH Exptl. (60)	293 ± 32	389 ± 58	487 ± 86	548 ± 97	539 ± 88	509 ± 101	538 ± 95
OH Contr. (30)	293 ± 32	318 ± 46	325 ± 60	311 ± 62	352 ± 87	323 ± 57	353 ± 59
L Exptl. (40)	132 ± 14	90 ± 17	59 ± 12	50 ± 13	86 ± 18	112 ± 15	129 ± 17
L Contr. (30)	132 ± 14	121 ± 17	123 ± 16	124 ± 13	131 ± 18	134 ± 18	128 ± 15

Days	4	6	10	14	30	45	60
OH Exptl.	601 ± 112	579 ± 106	593 ± 103	575 ± 105	520 ± 80	501 ± 98	521 ± 119
OH Contr.	340 ± 53	316 ± 58	316 ± 58	333 ± 66	316 ± 64	313 ± 51	307 ± 40
L Exptl.	129 ± 12	119 ± 9	124 ± 9	136 ± 19	127 ± 17	122 ± 18	134 ± 17
L Contr.	129 ± 12	119 ± 12	131 ± 17	134 ± 17	127 ± 17	131 ± 21	131 ± 14

Figures following ± are standard deviations.
Figures in parentheses are number of animals.
Doses are 2 mg. caffeine in 0.3 ml. distilled water for the obese; 5 mg. caffeine in 0.3 ml. distilled water for the nonobese.

weight). Unlike the situation with alloxan which was better tolerated after 24–48 hours of fasting,[5] deprivation of food increased toxicity of caffeine. In obese mice, survival rate, following administration of 2 mg. caffeine/animal after 24 hours fasting, was 72 per cent (18 of 25 injected animals survived); after 48 hours of food deprivation with the same dose of caffeine, only 10 per cent of obese animals survived (2 out of 20 injected animals). All lean caffeine-injected animals survived the injection of caffeine after 24 and 48 hours of fasting (lean animals were injected with 5 mg. caffeine/animal).

Effect of Caffeine on Blood Glucose

The effect of caffeine on blood glucose levels was remarkable. Sixty obese animals were injected with 2 mg. of caffeine in 0.3 ml. of distilled water, 40 lean animals with 5 mg. of caffeine in the same volume of distilled water. An obese-untreated group of 30 animals and nonobese-untreated group of 30 animals were injected with the same volume (0.3 ml./animal) of distilled water. Glucose determinations were done in duplicate by Somogyi-Nelson method modified for ultramicro determination[6] after 3, 6, 9, 12, 24 hours and 2, 4, 6, 10, 14, 30, 45, and 60 days following injection.

Lean animals showed an initial drop from an average level of 132 mg./100 ml. ± 14 to a lower level of 59 mg./100 ml. ± 12 after 6 hours and a minimum level of 50 mg./100 ml. ± 13 after 9 hours. Blood sugar values were back to normal after 24 hours and hereafter did not significantly differ from those of untreated animals.

In obese animals the blood glucose level rose from 293 ± 32 mg./100 ml. to 487 ± 85 mg./100 ml. after 6 hours, 539 ± 88 mg./100 ml. after 12 hours, and reached a peak of 601 ± 112 mg./100 ml. after 4 days. From there on, the blood glucose of the caffeine-injected obese animals remained, with minor fluctuations, in the range of 500–600 mg./100 ml. until the termination of the experiment, 60 days after injection. Blood sugar value was at that time 521 ± 119 mg./100 ml.

Glucose and Insulin Tolerance after Caffeine Treatment

A glucose tolerance test was conducted by injecting 50 mg. of glucose/animal intraperitoneally on the tenth day after injection of caffeine in 15 treated and 10 nontreated obese animals. At that time the treated group had blood sugars of 523 ± 92 and the untreated group 287 ± 42. Blood sugars failed to rise much in the caffeine-treated group (the 30 minute level was 595 ± 87, the 60 minute level was 574 ± 91, and the 120 minute level, 544 ± 98), while the nontreated group showed characteristically elevated glucose level curve. This failure of the caffeine-treated group to show a rise in blood sugar levels can probably be explained by the fact that in animals already having very high levels of glucose, additional glucose is very quickly spilled over in urine (a fact confirmed by urinalysis).

Insulin tolerance tests were conducted on the fifteenth day in 15 fed and 15 fasted (24 hour) obese mice and 10 fed and 10 fasted untreated animals; one unit of regular crystalline insulin was the dose used. Results confirmed

Table 2.—*Glucose Tolerance Test in Caffein-Treated and in Untreated Obese Hyperglycemic Mice*

	0	30'	60'	120'
OH Exptl. (15)	523 ± 92	595 ± 87	574 ± 91	544 ± 108
OH Contr. (10)	287 ± 42	425 ± 78	462 ± 92	303 ± 67

Figures following ± are standard deviations.
Figures in parentheses are number of animals.
50 mg. glucose administered to each animal.

Table 3.—*Insulin Tolerance in Nonfasted Caffeine-Treated and Untreated Obese Hyperglycemic Mice*

	0	30'	60'
OH Exptl. (15)	487 ± 71	412 ± 72	493 ± 87
OH Contr. (10)	304 ± 67	220 ± 46	297 ± 56

Table 3a.—*Insulin Tolerance in Caffeine-Treated and Untreated Obese Hyperglycemic Mice after 24-Hour Fast*

	0	30'	60'
OH Exptl. (15)	336 ± 74	325 ± 82	290 ± 62
OH Contr. (10)	128 ± 32	65 ± 17	55 ± 6

Figures following ± are standard deviations.
Figures in parentheses are number of animals.
1 I.U. reg. insulin/animal.

the previously established[7] resistance to inuslin of obese hyperglycemic mice, and do not indicate increased sensitivity to insulin after caffeine treatment.

Histologic Study

A group of 4 caffeine-treated and 4 nontreated obese animals was sacrificed on the day after caffeine injection. Histologic studies did not reveal the cause of the hyperglycemic effect of caffeine. The livers, adrenals, kidneys and pituitaries did not show any significant change. The pancreatic islets were even more degranulated than are those of the untreated obese hyperglycemic mice.* (We are indebted to Dr. S. B. Andrus for this histological examination of these animals.) The use of C-14 labeled caffeine (label in position 2) did not reveal incorporation of C-14 compound in the livers, pancreata, adrenals, pituitaries or kidneys of treated animals.

*The striking and apparently paradoxic effect of alloxan in this syndrome (permanent reduction of blood glucose and regranulation of pancreatic beta cells) has been reported previously.[6]

Effect of Coffee

Finally, in order to find out whether or not the ingestion of coffee would have effects similar to those of caffeine injection, 30 obese and 20 nonobese mice were given coffee as their exclusive source of fluid. Coffee was prepared daily from "instant" powder to insure greater reproducibilty than could be obtained by percolation. The solution contained 1.33 Gm. coffee and 2 Gm. sugar/100 ml. of distilled water corresponding to 0.33 mg. of caffeine/ml.* The animals were maintained on ad libitum intake of coffee and laboratory (Purina) chow for 7 days. Daily intake of coffee was measured. Daily intakes of coffee ranged from 2.5 to 14.0 ml. with an average of 6.2 ± 3.1 ml. Weekly intakes ranged from 21 to 58 ml. with an average of 43.5 ± 12.1 ml. After 7 days, the drinking fluid was changed back to water. Blood glucose determinations were carried out daily during the coffee ingestion and 3, 6, 10, 14, 21, 30, 45, 60, 75, 90, 92, 94, 96, 98 and 100 days after the animals stopped drinking coffee.

The blood sugar levels of the nonobese animals were not affected (average in the range of 100 to 135 mg./100 ml. for both water and coffee group). The blood glucose of the obese animals rose gradually while they were drinking coffee from 285 ± 29 mg./100 ml. to 395 ± 52 mg./100 ml. on the sixth day. Coffee was discontinued after 7 days and the animals were allowed to rest for 3 days in order to excrete most of the caffeine to enable us to establish the "late" effect of coffee intake. Blood sugar values continued to rise steadily to reach their peak 7 days after coffee had been discontinued. At that time, the value was 537 ± 82 mg./100 ml. Blood sugar values remained elevated, with minor fluctuations, in the range of 480–550 mg./100 ml. until the 75th day of the experiment (or 68 days after coffee had been discontinued). The next blood glucose determination was done on the 90th day of the experiment and at that time, we found that blood sugar values had fallen to a very low level (128 ± 37 mg./100 ml.) From that day on, blood sugar values were checked every 2 days through day 100. At that time, the experiment was discontinued because in the last 5 days of the experiment, 18 of the remaining 24 animals had died. The average blood sugar level on day 100 was 108 ± 54 mg./100 ml. It might be of interest to report that the blood sugar levels in the animals drinking coffee appeared to rise in direct proportion to the amounts of coffee they had drunk (and hence of the caffeine they ingested), p being less than 0.01.

Other Compounds

In order to find out whether or not this specific glucotropic effect of alloxan and/or caffeine is due to their specific chemical structure, we tested, on 10 animals each, other alloxan-like and caffeine-like compounds (uric acid, lactin and lactan, adenine, cytosine, guanine, hypoxanthine, uracil, thymine

*We are grateful to Mr. Nevis Cook, of the Food and Drug Administration for the determination of the caffeine content of the type of instant coffee used in this experiment.

Table 4.—*Long-Term Effect on Blood Glucose Levels of Replacement of Water by Coffee for 1 Week on the Blood Glucose of Obese Hyperglycemic Mice*

Days	0	3	6	10	14	30	45
OH Exptl. (30)	285 ± 29	360 ± 47	395 ± 52	417 ± 76	537 ± 82	486 ± 97	504 ± 72
OH Contr. (20)	285 ± 29	265 ± 39	288 ± 60	248 ± 51	294 ± 48	302 ± 61	298 ± 47

	60	75	90	92	94	96	98	100
OH Exptl.	527 ± 99	509 ± 102	128 ± 37	135 ± 42	108 ± 32	96 ± 47	114 ± 34	108 ± 54
OH Contr.	318 ± 72	277 ± 64	286 ± 42	304 ± 59	279 ± 34	299 ± 59	308 ± 48	302 ± 60

Figures following ± are standard deviations.

Figures in parentheses are numbers of animals.

0 day is the beginning of the experiment, and the beginning of replacement of water by coffee. The initial glucose determination was conducted before any coffee was drunk.

and xanthine) but found no significant long-term changes in blood sugar levels with any of these compounds. The only one to show some temporary rise in blood sugar levels was hypoxanthine where blood sugars rose from an initial 302 ± 47 mg./100 ml. to 397 ± 54 mg./100 ml. on the third day after 4.2 mg./animal injection of hypoxanthine. Blood sugar levels rose further on the fifth day to 422 ± 68 mg./100 ml., but after 7 days after injection, they were back to normal, i.e., 310 ± 24 mg./100 ml. and remained in that range for 3 more weeks, at the end of which period, the experiment was discontinued.

Discussion

It is intriguing to note that in obese-hyperglycemic mice, caffeine in many ways has an action opposite to that previously described for alloxan. Further work is obviously necessary to establish the mechanism of this unexpected action of caffeine and the possible relevance of these findings to the management of maturity-onset diabetes in obese human subjects. The word *possible* should be underlined: we know at present of no evidence bearing on the effect of caffeine or coffee in human diabetes, but in view of the large consumption of coffee, this is obviously a subject which deserves further study.

REFERENCES

1. Mayer, J.: The obese hyperglycemic syndrome of mice as an example of "metabolic" obesity. Am. J. Clin. Nutrition 8:712, 1960.
2. Christophe, J., Jeanrenaud, B., Mayer, J., and Renold, A.: In vitro metabolism of adipose tissue in obese hyperglycemic and goldthioglucose treated mice. II. Metabolism of pyruvate and acetate. J. Biol. Chem. 236:648, 1961.
3. Leboeuf, B., Lochaya, S., Leboeuf, N., Wood, F., Mayer, J., and Cahill, G.: Glucose metabolism and mobilization of fatty acids by adipose tissue from obese mice. Am. J. Physiol. 201:19, 1961.
4. Treble, D., and Mayer, J.: Glycerokinase Activity in white adipose tissue of obese hyperglycemic mice. Nature 200:363, 1963.
5. Solomon, J. B., and Mayer, J.: Effect of alloxan on obese hyperglycemic mice. Nature 192:135, 1962.
6. Natelson, C. C.: Nelson-Somogyi Ultramicro method in: Micro Techniques in Clinical Chemistry, 2nd ed. Springfield, Ill., Thomas, 1961, pp. 218–219.

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The Mutagenic Action of Caffeine in Higher Organisms[1]

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Introduction

Caffeine is a purine, 1, 3, 7-trimethyl xanthine. Since it is consumed in great quantities by man, the question concerning whether it is a mutagen for man is of great significance. There is now good evidence that caffeine is mutagenic not only in bacteria (4, 5, 7, 9, 18), fungi (6, 41), and plants (11, 12), but equally mutagenic in *Drosophila melanogaster* [2, 16, 25, 29; negative reports, (40)], in mice [Kuhlmann and Ostertag 1968, unpublished; negative report, (15); ambiguous results, (3)], as well as in human cells *in vitro* (24, 26, 28; Jacobson, unpublished). There is some evidence that caffeine inhibits dark repair in bacteria (38) as well as in mammalian cells (33; Jacobson, C., unpublished). Caffeine is teratogenic in mice if high doses are applied (17). There is no evidence that caffeine is involved in carcinogenesis. We have good evidence from studies with *Drosophila melanogaster* that caffeine also causes somatic damage by breakage of chromosomes in somatic cells (29). By analogy with the action of radiation, which causes the same type of somatic damage (23; Ostertag, unpublished data), we have good reason to assume that caffeine induces premature aging in Drosophila.

Effect of Caffeine on Cell Growth and Nucleic Acid Metabolism in Mammalian Cells

Caffeine inhibits the growth of HeLa cells in concentrations above 200 μg/ml (26). At the same concentration we find inhibition of RNA synthesis, but not of DNA synthesis (Chart 1). At very low doses there is possibly a stimulation of DNA synthesis. By following the kinetics of the inhibition of DNA and RNA synthesis at a dose of 500 μg/ml, DNA synthesis proceeds normally up to 20 hours, while inhibition of RNA synthesis starts at about 2–3 hours after caffeine application and is then about 50% (Chart 2) (Heege, E. M., unpublished results). At these dose levels of caffeine we would therefore expect a change in the ratio of DNA/RNA. We do not know whether the synthesis of any of the three major RNA species transfer RNA, messenger RNA (mRNA), ribosomal RNA is preferentially inhibited. Recently, inhibition of DNA polymerase activity by caffeine has been observed in human cells in an *in vitro* assay at concentrations above 500 μg/ml after 48 hours. If mitomycin C or aflatoxin B are added to these cultures, DNA polymerase activity is elevated at certain concentrations by about 50%. This elevation of DNA polymerase activity is inhibited by caffeine concentrations above 200 μg/ml (39). Recently we checked the period of DNA synthesis after exposure of HeLa cells to caffeine. In HeLa cells one division cycle normally lasts 20.5 ± 1 hr. DNA synthesis (S phase) takes 6 hours; a lag phase then follows without DNA synthesis (G-2) for approximately 4.6 hr. before mitosis, which takes 1.1 hr. The rest of the division cycle is the G-1 phase (32). We added 1% caffeine for two hours at 0 time, removed the caffeine, and then exposed the cells to thymidine-^{3}H for 10 minutes at various times after caffeine exposure in order to label the newly synthesized DNA. Twenty-four hours after 0 time, the cells were fixed and autoradiographs were prepared. For each metaphase we determined whether there was any label incorporated into the chromosomes. Exposure to these high concentrations of caffeine for two hours affects the cell cycle only slightly. The time between DNA synthesis and the next metaphase is about one hour longer than in the untreated cells (Heege, E. M., unpublished results) (Chart 3).

Morphology of Cells Treated with Caffeine

HeLa cells lose their attachment to the glass of the Petri dishes if treated with more than 100 μg/ml of caffeine. They also get more spherical as if they had been treated with trypsin (Figs. 1, 2). If 1% caffeine is used, there are extensive changes of the fine structure of HeLa cells (27). The main damage is to the nucleus, which starts to segment; nuclear fragments move across the cytoplasm and are found outside the cell membrane. This expulsion of nuclear material is effected only by caffeine and has not been found after exposure to other damaging influences. It is perhaps similar to the enucleation process during normal erythrocytic maturation (Figs. 3–5). The extrusion of nuclear fragments might be a consequence of the unbalanced nucleic acid metabolism and the relative lack of mitotic inhibition which is found after caffeine exposure. Agents such as actinomycin D, mitomycin C, and chromomycin A3, which also induce imbalance of nucleic acid synthesis (in one way or another), are very potent inhibitors of mitosis in mammalian cell cultures (25), yet they do not induce expulsion of nuclear fragments.

By high resolution autoradiography we could show that those cells which respond to caffeine application by fragmentation of their nuclei have lost their ability to incorporate thymidine-^{3}H. In the same culture there are other cells which incorporate thymidine-^{3}H to the same extent as in control

[1] This research was supported by the Landesamt für Forschung, Land Nordrhein-Westfalen.

[2] Reprint requests should be addressed to Dr. Ostertag.

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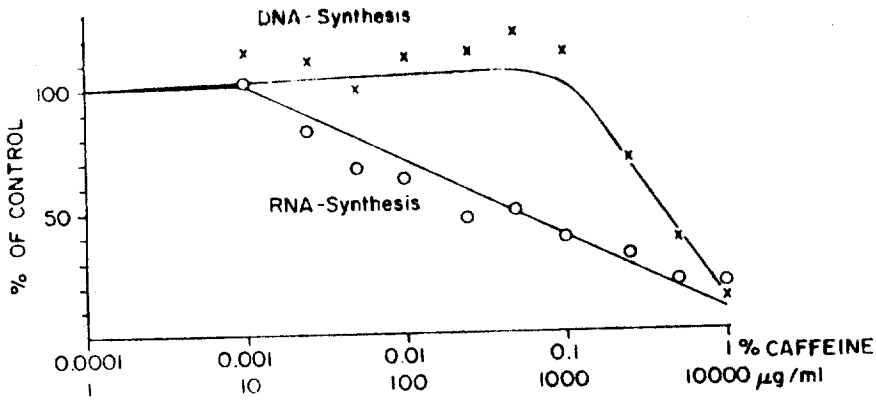

Chart 1. Inhibition of DNA/RNA synthesis by caffeine.

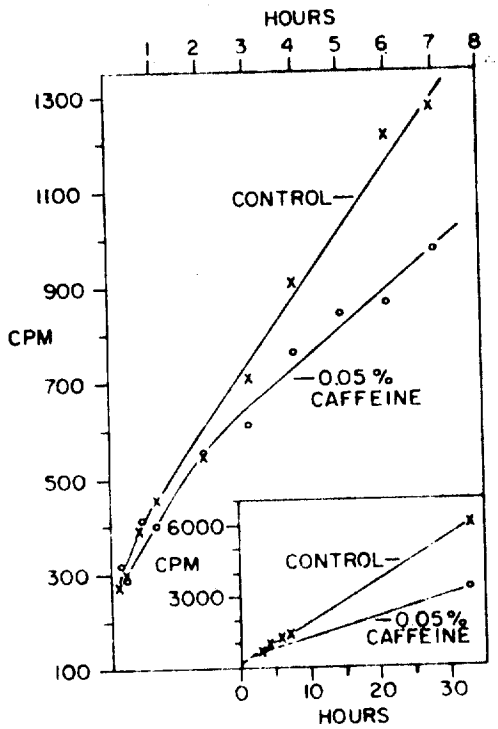

Chart 2a. Inhibition of RNA synthesis by 0.05% caffeine (^{14}C orotic was added at time 0).

experiments (Fig. 6). After caffeine treatment almost all of the DNA is perinuclear. This has also been found after X-irradiation of mammalian cells (Figs. 7, 8).

Chromosome Breakage by Caffeine

Type of Breaks Induced by Caffeine. As can be seen in Figs. 9 and 10 and Table 1, caffeine, when applied to human cells in culture, does not induce many translocations, rings, and other rearrangements that require fusion of the broken ends. The rearrangements, if present, are often in clusters in one single cell. We therefore have good reason to assume that these rare rearrangements are induced by a secondary, physiologic mechanism. We conclude that caffeine, in contrast to all other agents that cause chromosome or chromatid breakage, does not induce such sticky breaks. The rejoining mechanism, which is possibly part of the repair mechanism, seems to be blocked for caffeine-induced chromatid breaks. In addition to normal breaks, we quite often find lesions of a long stretch of chromatin. These cannot be caused by two breakage events, as nonterminal deletions normally are, but rather must be caused by a failure of synthesis, possibly combined with enzymatic removal of the DNA strand that failed to replicate.

Dose Response. Caffeine does induce breaks in human chromosomes even in doses below 500 µg/ml (Chart 4, Table 1). Various doses were applied for one hour. The caffeine-containing medium was then removed, the cells were washed with caffeine-free medium, and caffeine-free medium was added for another 23 hours. The cells were then fixed and the metaphases examined. There is an increase of the caffeine-induced breakage correlated to the dose. This increase is linear in the lower dosage ranges and more than linear at very high doses. To see whether the greater than linear increase in breakage at higher dose level was due to the same mechanism that induced breakage at a lower dose of caffeine, we analyzed cells with similar chromosome number for the number of breaks per cell at the higher dose level.

We would expect that there is a very high frequency of breakage in the same cell if at a certain threshold a physiologic chain of reactions is initiated that results in secondary chromosome breakage, perhaps due to release of DNases. We therefore would expect two types of cells, those showing a very high frequency of breakage and those having much less breakage. The latter type would be linearly dose dependent, and the former would be absent at a low dosage. Corresponding to expectation, we found that 1% caffeine exposure caused a deviation in the distribution of breaks per cell from the average number of breaks expected according to the Poisson distribution (Chart 5). There are many more cells with a high number of breakage per cell than expected (25 breaks and more per cell; expected 0.45%, found 13.75%). The dosage

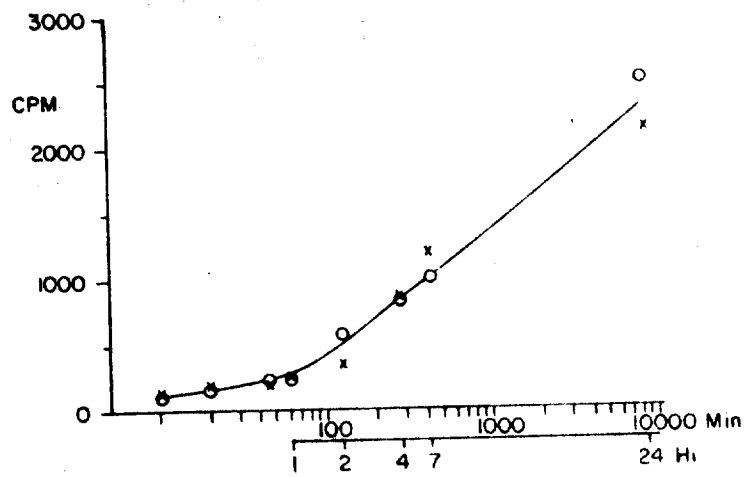

Chart 2*b*. Lack of inhibition of DNA synthesis by 0.05% caffeine, (Thymidine-^{14}C was added at time 0). -o-o-o-, control; -x-x-x-, 0.05% caffeine.

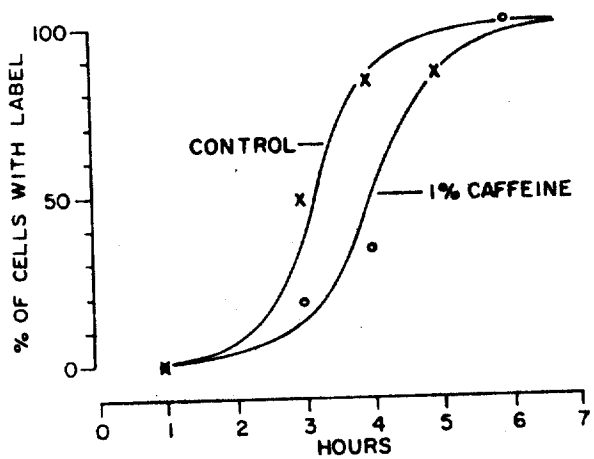

Chart 3. Time between DNA synthesis and metaphase.

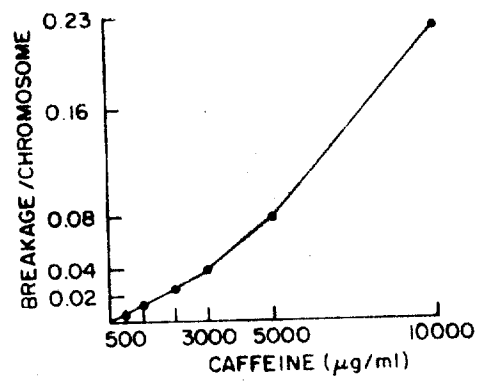

Chart 4. Breakage induced by caffeine in HeLa cells. Dose dependence [Ostertag *et al.* (26)].

Table 1

Caffeine (μg/ml)	Number of chromosomes	Chromatid + isolocus breaks: Number	Chromatid + isolocus breaks: per chromosome	Translocations	Dicentrics	Rings
0	4187	4	0.0009		1	
500	2754	18	0.0065		2	
1000	3693	47	0.0128	*a*		
2000	4361	111	0.0250	1		
3000	2663	106	0.0398	1	2	
5000	5666	401	0.0708	5	2	3
10,000	3456	763	0.221	11	3	

Number of breaks induced by caffeine per chromosome. Chromosome analysis 24 hr after 1-hr exposure. Reproduced from Ref. 26.

a 2 cells with 10 translocations.

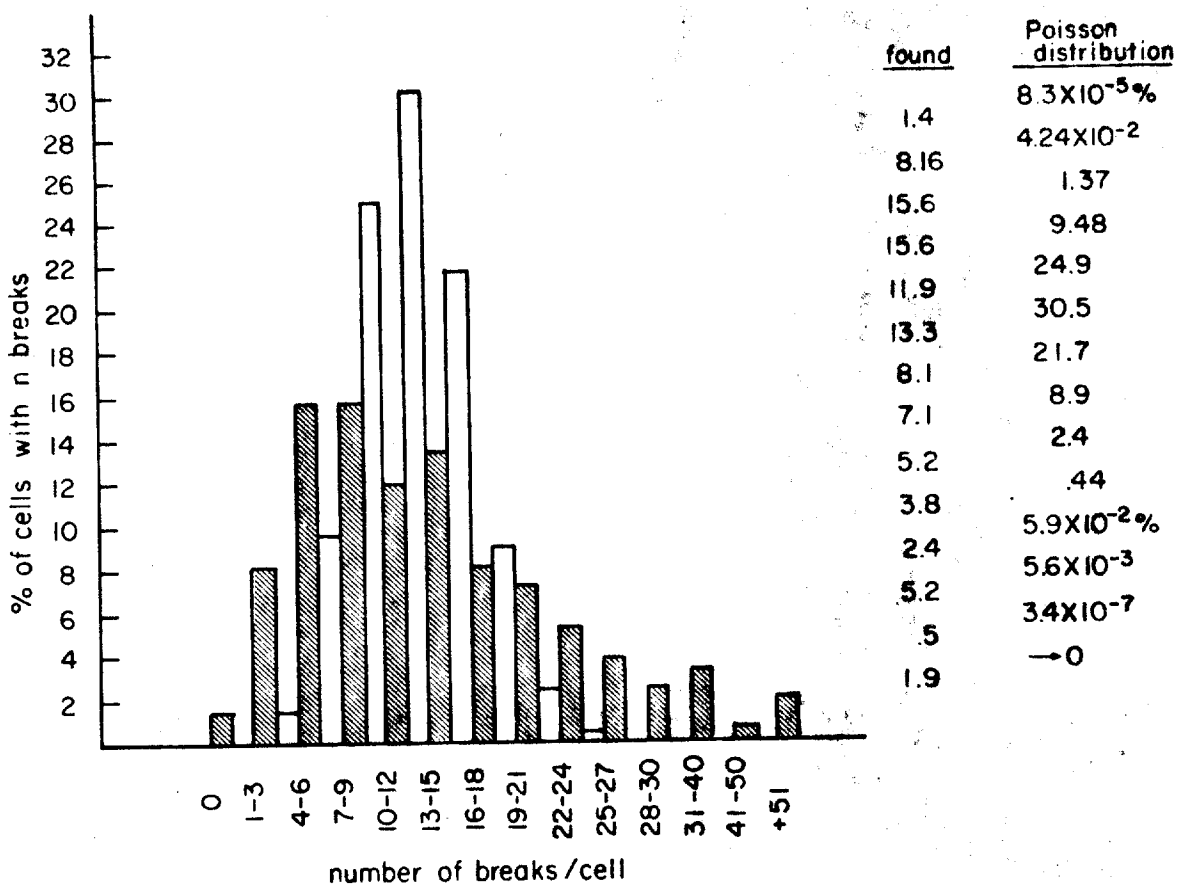

Chart 5. Number of breaks/cell using 1% caffeine, ☐, found; ▨, expected.

response is linear if we exclude these cells with an abnormally high number of breaks even at the higher dosage (1%, 0.5% caffeine). The distribution of breaks per cell of the remaining cells corresponds now to the Poisson distribution which would be expected if caffeine-induced damage is due to a single-hit phenomenon.

Time of Action during the Cell Cycle. Caffeine even in high doses affects the cell cycle of HeLa cells so slightly that it can be neglected. To test the action of caffeine on the various stages of the cell cycle, we treated the cells with caffeine for 1.5 hours at different intervals before fixation. The results are shown in Chart 6. Apparently the caffeine-induced breakage is realized only during DNA synthesis, since treatment of cells with caffeine in the G-2 phase does not induce breakage in the first mitosis after *treatment*, but in the first mitosis after *DNA synthesis*. The average probability of breakage induction for any period of the cell cycle seems to be the same (24). This is different for breaks induced in leukocyte cultures (28). The dependency of breakage on replication suggests that caffeine-induced breaks are not closely dependent on the total time of exposure if treatment is during one cell cycle before DNA replication. We tested this

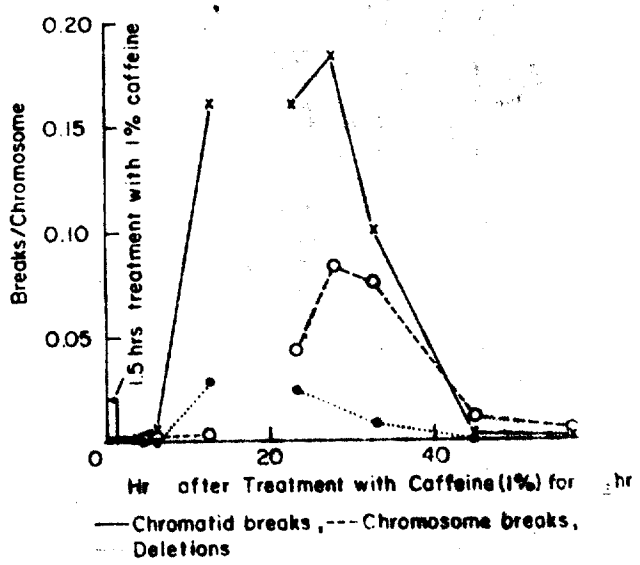

Chart 6. Breakage of chromosomes by caffeine in relation to the time of treatment during the cell cycle. HeLa cells [Ostertag (24)].

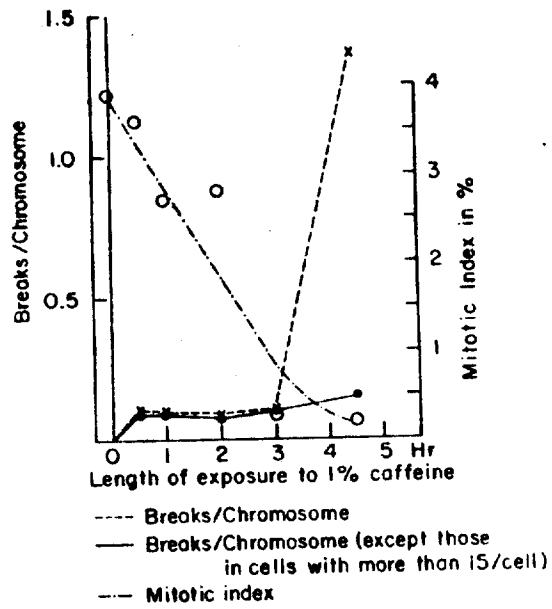

Chart 7. Dependence of caffeine-induced breakage on total time of exposure to caffeine. HeLa cells. [Ostertag (24)].

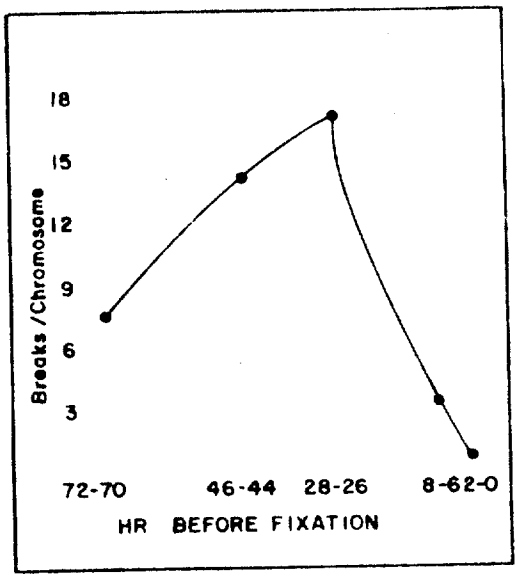

Chart 8. Breakage of chromosomes by caffeine in relation to the time of treatment during the cell cycle. Cells in leukocyte culture. (1% caffeine, 2-hr treatment).

question using 1% caffeine and varying lengths of exposure (Chart 7). The predication that caffeine-induced breakage is only dependent on dosage was shown to be true for exposure times up to four hours. After this, extensive, irreversible cell damage can be observed. This is probably the cause of the increase in the total amount of breakage after 4 hours treatment with 1% caffeine. Apparently a secondary physiologic kind of damage, probably the same as described above, occurs at high doses and longer exposure which leads to nonspecific breakage (24). Caffeine-induced breakage dependent on DNA synthesis could also be demonstrated by our results with human leukocyte cultures (28) (Chart 8). Breakage in these cells is *most frequent* if the cells are treated in middle and late G-1 phase, low in late DNA synthesis period, and *almost absent* if treated during G-2. If treatment is in early G-1, the breakage is also relatively low. This could be due to the as yet low rate of mRNA synthesis during early G-1 shortly after stimulation with phytohemagglutinin. Ts'o *et al* (36, 37) previously have shown that caffeine forms complexes with DNA, both with the coil, and to a lesser extent, with the helix DNA. DNA is probably in the coil or single-stranded form during mRNA synthesis at the site of mRNA synthesis. Razin (34) has found single-stranded DNA by means of immunofluorescent antibody staining in lymphocyte chromosomes throughout the cell cycle. If caffeine binds mainly at the sites of mRNA synthesis, one should observe less breakage after treatment in a period with a low amount of mRNA formation. This has been found.

Chromatid Breakage in Leukocyte Cultures. Caffeine induces breakage not only in HeLa cells, but also in short term leukocyte cultures. The breakage per chromosome is closely correlated with the measured physical length of the same chromosomes if treatment is before DNA synthesis in G-1. This is no longer true if treatment is in late S phase towards the end of DNA synthesis, as can be shown by plotting the distribution of breaks. Instead there is much reduced breakage, predominantly in late replicating chromosomes (B chromosomes, and perhaps one of the X chromosomes of the female cells) (Chart 9). Presumably, the late replicating chromosomes would be underrepresented if one could also score the next mitosis, and all other chromosomes would probably be overrepresented. These results show again the correlation of DNA replication and chromosome breakage by caffeine (28). If we plot the distribution of breakage per different chromosome regions, we observe a marked deviation from expectation (28). Centromere regions show less and telomere regions more breakage than expected. This agrees with the distribution of breakage found after treatment with bromodeoxyuridine (10), but is in contrast with the spectrum of breakage after exposure of mammalian cells to actinomycin D (24, 30) and hydroxylamine (35). Actinomycin D and hydroxylamine preferentially induce breakage in the centromere regions. A correlation of the breakage spectrum to base composition of the DNA seems premature and unwarranted.

Action of Caffeine on Mice. Lyon *et al.* (15) have found no significant increase in mutation rate ("single locus test" for recessive mutations) after treatment of male or female mice with caffeine. The expected mutation rate, if the amount of breakage can be taken as a measure for gene mutation, should, however, be in the neighborhood of the natural mutation rate with the doses used by Lyon *et al.* Their data are not sufficient to exclude such a possibility. Moreover, if the mutagenic specificity of caffeine is unique, as suggested by the ability to induce breaks that have lost the capacity to rejoin, we might not find the type of mutations Lyon *et al.* were looking for. In another earlier study, Cattanach (3) showed that there is a

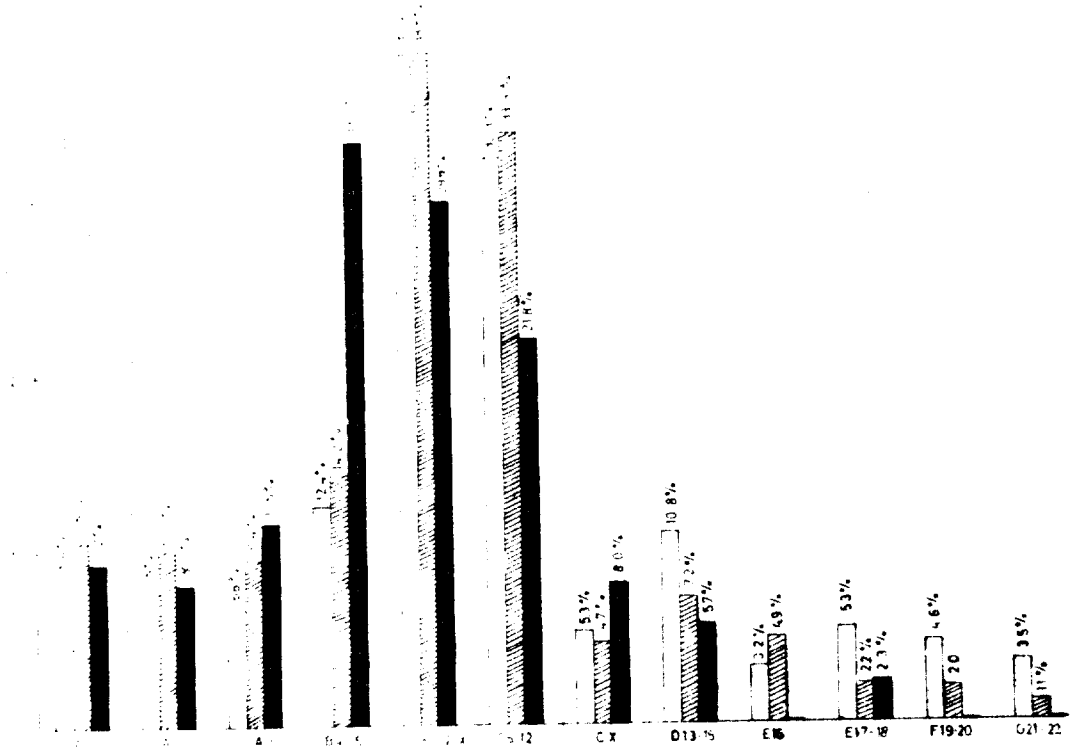

Chart 9. Distribution of caffeine-induced breaks [ ] relative length of chromosomes; ▨, breakage/chromosome in % treatment in G1 ■ breakage/chromosome in % treatment in late S phase. Abscissa chromosome number; ordinate, percent.

distinct decrease in fertility (which might in part be due to induced dominant lethals acting before implantation of the blastocyst) if male mice are treated with caffeine. These results were recently confirmed by us and extended by the group of Dr. Legator (unpublished results) at the FDA using rats. Even at dosage levels corresponding to 3 cups of coffee in man, there is a significant reduction of fertility if male rats are treated over a longer period. Cattanach noticed some cytologic aberrations in mice after caffeine treatment, but generally he concluded, "Without assumptions *ad hoc*, such as the production of breaks without rejoining ability, it is indeed difficult to see how a chemical that produces dominant lethality through chromosome breakage should fail to produce at least some viable rearrangements." We have shown that this is indeed the case; caffeine does induce breaks without ability to rejoin. We therefore reinvestigated the possibility of caffeine as a mutagen in mice. Since caffeine-induced breaks are only realized during replication or repair [according to our results and results obtained with bacteria (14)], we could not expect any pronounced effect on female germ cells since, in those, DNA replication is terminated after birth. We therefore exposed C57BL male mice to caffeine in their drinking water at various concentrations ranging from 0.025 to 0.5%. To get an accumulation of genetic damage, we treated for a longer period of time—on the average, 100 and 140 days. With each replication the caffeine-induced damage should increase by a constant factor. The treated males were then mated to C3H females, usually after exposing the treated males for three weeks or more to drinking water free of caffeine to eliminate any possible physiologic effect on the developing sperm cells. The preliminary results (Table 2) show that caffeine does induce dominant lethal mutations as indicated by (*a*) preimplantation losses and (*b*) resorptions, inviable embryos that can be seen after sacrificing and dissecting the pregnant female before term. If both types of dominant lethals are pooled, we get an initially linear increase of dominant lethals with caffeine dose (Chart 10).[3] The increase is less than linear at higher doses even if the results are corrected for doubly lethal hits using the Poisson distribution. The data are, however, not sufficient to establish a good dosage relationship. We conclude that caffeine is mutagenic for mammals not only *in vitro* but also *in vivo* (Kuhlmann and Ostertag, unpublished results). At the present time, Röhrborn at the Institute for Human Genetics in Heidelberg is checking whether dominant lethals in mice are induced at dosage levels corresponding to those to which humans are exposed.[4]

Action of Caffeine on *Drosophila melanogaster*. There are two conflicting reports on the possible mutagenic action of

[3] In each of the caffeine exposed series we have found a clustering of dominant lethals. There is still a significant increase in dominant lethals if these clusterings are excluded.

[4] Dr. Röhrborn has now found some effects of caffeine using inbred strains of mice, but never little of heterozygous males were used (personal communication).

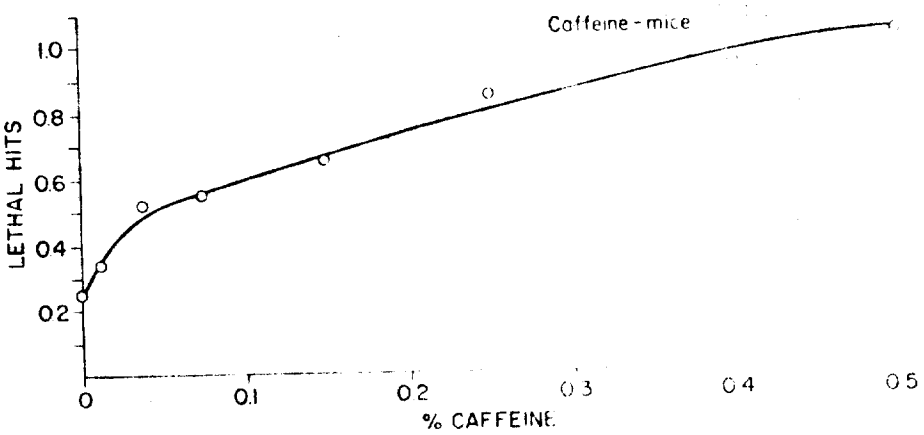

Chart 10. Dominant lethals induced by caffeine in mice. Correction applied. Poisson distribution.

caffeine on Drosophila in the earlier literature. Andrew (2) reported a significant increase of recessive lethal mutations after exposure of male Drosophila to caffeine. Yanders and Seaton (40) were unable to reproduce these results. They observed only a very slight, if any, effect of caffeine. This increase would be somewhat higher if they had included a cluster of mutations in the caffeine-treated sample. This correction is not statistically proper. In view of these results we decided to reinvestigate this question. We exposed the female larvae to 1% caffeine in a mixture of sugar, yeast, and water for one hour. They were then returned to their normal milieu, where they were allowed to hatch. We were able to observe a change in sex ratio (sex ratio is here defined as % of males in the total number of flies) in favor of the females. That is, more male than female larvae died after caffeine exposure. In the control we had 52.6% males, and in the caffeine-treated sample, 47.1% males. The average survival for the animals exposed to caffeine was 60.6%. The shift of sex ratio in favor of the females is highly significant ($P = 0.01$). Previously Oster (21), Ostertag and Muller (31), and Ostertag (23) have shown, for X-rays as well as for chemical mutagens (23), that this differential somatic mortality is due to genetic damage induced by chromosome breakage. Loss of the X chromosome in male cells in larvae presumably leads to cell death, whereas loss of one of the two X chromosomes in female cells can be tolerated. The same differential mortality, if larvae are treated with X-rays, also holds for the time after hatching. That is, X-rays induce premature aging. Premature aging can be traced back to loss of chromosomes in somatic cells (23; unpublished data). It is quite likely that caffeine induces premature aging as well. If the treated females are crossed to suitable males that are untreated, we find an increase of XO males due to chromosome loss in the progeny (F_1). The increase is in one cross from 0.15% of the controls to 0.50% of the treated animals, in a different cross, from 0.34% to 78%. There is no increase, and, in fact, possibly a decrease, in nondisjunction. The sex ratio is also changed in the progeny in favor of the females. This is an indication that caffeine does induce dominant sex-linked lethals. These results thus clearly demonstrate a significant increase in chromosome loss via chromosome breakage due to caffeine in Drosophila (29). We also tested whether caffeine induces sex-linked recessive lethals.

Table 2

Caffeine concentration[a] (%)	Matings	Pregnant animals	Corpora lutea[b]	Implantation	Living embryos	Preimplantation losses No.	Preimplantation losses %[c]	Implant losses No.	Implant losses %[d]	Total loss %[c]
0–.5	10	8	54	41	35	13	24	6	14	35
	11	10	82	55	44	27	34	11	20	46
	16	14	120	88	74	32	27	14	16	38
	15	8	68	36	29	32	47	7	19	57
	16	11	83	56	36	27	33	20	36	57
	13	10	74	41	27	33	43	14	34	64
0.25–0.5	81	61	481	317	245	164	34	72	22	49
[illegible]tr.	11	8	64	52	50	12	19	2	4	22

[illegible]ominant lethals induced by caffeine in mice.
[illegible]reated for 100 days; 0.2 and 0.4% for 140 days.
When the Corpora lutea could not be counted unambiguously, the average number 8 (377:47) has been used.
% of total number of Corpora lutea.
% of total number of implantations.

For this purpose we crossed individual F_1 females carrying one treated and marked and one untreated chromosome to males with a normal X chromosome. In the progeny (F_2) we counted those crosses with only one type of males hatching (recessive lethal for the chromosome not represented in the progeny). There is a significant increase in sex-linked recessive lethals from 0.32% of the controls to 2.48% for the caffeine-treated series (Table 3). Our results with Drosophila indicate that caffeine induces (*a*) somatic damage (and premature aging), (*b*) chromosome breakage, (*c*) dominant lethality, and (*d*) recessive lethal mutations (29). Similar results with male flies being treated with caffeine have now been reported by Mittler (16). There are some minor discrepancies between his and our results, but they need not be discussed.

Table 3

	Chromosomes tested	Sex-linked recessive lethals		
	Σ	Σ	%	P
Control	4,420	14	0.32	1
Mitomycin C	1,066	13	1.22	0.0004
Caffeine	774	19	2.48	$<10^{-10}$
Actinomycin D	408	1	0.25	0.82

Sex-linked recessive lethals, cross 2 (F_2). Reproduced from Ref. 29.

Discussion and Summary

Summarizing our main results and those of others working with caffeine as a mutagen [and both ethoxy-caffeine, (Ostertag, unpublished) and theophylline, which is one-tenth as effective as caffeine] we can conclude:

(*a*) Caffeine is mutagenic in Drosophila. It induces dominant lethals in mice if male mice are treated chronically.

(*b*) It induces chromatid breakage in human cells *in vitro*.

(*c*) The breaks do not tend to rejoin. Rearrangements are usually absent.

(*d*) The breakage is, in lower dosage ranges, linearly dependent on dose. One would thus expect that lower doses would induce breakage (mutations) in proportion to the dose.

(*e*) The caffeine-induced breakage is realized only during DNA synthesis. However, premutations can be induced in roughly the same amount throughout the cell cycle. The breakage is not dependent on the total time of exposure to caffeine. It is therefore likely, if the same is true for caffeine induced mutations in man, that caffeine induces only minimal genetic damage in nondividing tissue. Since in women the DNA replication of germinal cells is terminated with birth, almost no genetic damage would be induced in women after birth. Caffeine-induced mutagenic damage, however, could accumulate in men.

(*f*) Caffeine induces somatic damage due to chromosome breakage and loss and probably induces premature aging in Drosophila.

(*g*) Caffeine inhibits DNA dark repair as evidenced by the interference with the repair of UV-induced damage in mouse L cells (33), by the enhancement of X-ray-induced chromosome damage in leukocyte cultures (C. Jacobson, personal communication), and by the inhibition of the mitomycin C-induced increase in DNA polymerase activity in human cell cultures (39). Oxygen enhancement of caffeine-induced chromosome breakage as found in ascites tumor S_2 sarcoma in mice by Adler and Schöneich (1) can perhaps also be explained on that basis.

We would like to discuss a tentative model of the action of caffeine on a molecular basis. The model would explain both effects on repair mechanisms and induction of mutations by caffeine itself. We assume that caffeine forms complexes with DNA *in vivo* as it does *in vitro* (36, 37). This complex binding leads to inhibition in the repair of previously nicked DNA. If during replication there is only one DNA polymerase adding nucleotides in 5′–3′ direction on the new strand that is being replicated (13), we are forced to conclude either that (*a*) only one strand is synthesized in one piece in 5′–3′ direction while the other is synthesized piecewise also in 5′–3′ direction but in the opposite direction to the first strand in relation to the axis of the DNA, and only from the place of replication of the first strand to the already incompletely replicated piece of the second strand, or (*b*) that both newly synthesized strands are synthesized in small pieces. In this latter case the DNA replication would jump from the one strand to the other and would work its way backward on the other strand. The replication would then always proceed in small pieces in 5′–3′ direction which would have to be joined by a DNA ligase as occurs during repair of nicked DNA. Evidence for a piecewise replication of DNA has recently been obtained by Okazaki *et al.* (19, 20). Their results favor the piecewise replication of both strands. During both repair and normal replication, a joining of nicked or unconnected DNA strands would be necessary, which might be inhibited by caffeine. There are several possible ways that caffeine could inhibit such a process, e.g., by reacting with the joining enzyme, or by being incorporated terminally into the DNA. If this is the action of caffeine, the two aspects of caffeine action, inhibition of dark repair, and mutations realized only during replication could be due to the same basic mechanism. If the possible genetic damage by caffeine in man is estimated using the results of our experiments with human cells in culture and *Drosophila*, and assuming that caffeine is distributed evenly throughout the body, which seems to be true for the testes in men and for the germ cells of the embryo (8), we obtain a calculated total amount of genetic damage due to caffeine in the neighborhood of the natural mutation rate. One cup of coffee would then be equivalent to 0.01 R X-irradiation in men but only a fraction (1/26) of that in women (24). There is thus a strong likelihood that caffeine may prove to be one of the most dangerous mutagens in man. Further experiments using caffeine with other mammals are now in progress at several places and might lead to a more certain conclusion whether caffeine is really mutagenic in man. Data on the sex ratio of the F_2 generation in man following consumption of caffeine or theobromine in the grandparental generation should also be sought. Furthermore, experiments should be conducted to examine whether caffeine accelerates aging, induces malignant transformations, or acts as a cocarcinogen by inhibiting repair processes.

REFERENCES

1. Adler, I. D., and Schöneich, J. Mutagenic Action of Caffeine in an Ascites Tumour Strain of Mice: Cytogenic Investigation. Humangenetik, *4:* 374–376, 1967.
2. Andrew, L. E., The Mutagenic Activity of Caffeine in *Drosophila.* Am. Naturalist, *43:* 135–138, 1959.
3. Cattanach, B. M. Genetical Effects of Caffeine in Mice. Z. Vererbungslehre, *93:* 215–219, 1962.
4. Demerec, M., Bertani, G., and Flint, J. A Survey of Chemicals for Mutagenic Action on *E. coli.* Am. Naturalist, *85:* 119–136, 1951.
5. Demerec, M., Witkin, E. M., Catlin, B. W., Flint, J., Belser, W. L., Dissosway, G., Kennedy, F. L., Meyer, N. C., and Schwartz, A. The Gene, Carnegie Inst. Wash. Publ., *49:* 144–151, 1950.
6. Fries, N. The Production of Mutations by Caffeine. Hereditas, *36:* 134–149, 1950.
7. Gezelius, K., and Fries, N. Phage Resistant Mutants Induced in *E. coli* by Caffeine. Hereditas, *38:* 112, 1952.
8. Goldstein, A., and Warren, R. Passage of Caffeine into Human Gonadal and Fetal Tissue. Biochem. Pharmacol., *11:* 166–168, 1962.
9. Greer, S. B. Growth Inhibitors and Their Antagonists as Mutagens and Antimutagens in *Escherichia.* J. Gen. Microbiol., *18:* 543–564, 1968.
10. Hsu, T. C., and Somers, C. E. Effect of 5-bromodeoxyuridine on Mammalian Chromosomes. Proc. Natl. Acad. Sci. U. S., *47:* 396–403, 1961.
11. Kihlman, B. A. A Survey of Purine Derivatives as Inducers of Chromosome Changes. Hereditas, *38:* 115–127, 1952.
12. Kihlman, B. A., and Levan, A. The Cytological Effect of Caffeine. Hereditas, *35:* 109–114. 1949.
13. Kornberg, A. Biosynthesis of DNA. In Koningsberger, V. V. and Bosch, L. (eds), Regulation of Nucleic Acid and Protein Synthesis, pp. 23–38. Amsterdam, London, New York: Elsevier Publ. Co. 1967.
14. Kubitschek, H. E., and Bendigkeit, H. E. Mutation in Continuous Cultures, I. Dependence of Mutational Response upon Growth-limiting Factors. Mutation Res., *1:* 113–120, 1964.
15. Lyon, M. F., Phillips, J. S. R. and Searle, A. G. A Test for Mutagenicity of Caffeine in Mice. Z. Vererbungslehre, *93:* 7–13, 1962.
16. Mittler, S., Mittler, J. E., and Owens, S. L. Loss of Chromosomes and Nondisjunction Induced by Caffeine in *Drosophila.* Nature, *214:* 424, 1967.
17. Nishimura, H., and Nakai, K. Congenital Malformations in Offspring of Mice Treated with Caffeine. Proc. Soc. Exptl. Biol., *104:* 140–142, 1960.
18. Novick, A. Mutagens and Antimutagens. Brookhaven Symp., *8:* 201–215, 1955.
19. Okazaki, R., Okazaki, T., Sakabe, K., and Sugimoto, K. Mechanism of DNA Replication. Possible Discontinuity of DNA Chain Growth, Japan. J. Med. Sci. Biol., *20:* 255–260, 1967.
20. Okazaki, R., Okazaki, T., Sakabe, K., Sugimoto, K., and Sugino, A. Mechanism of DNA Chain Growth, I. Possible Discontinuity and Unusual Secondary Structure of Newly Synthesized Chains, Proc. Natl. Acad. Sci. U. S., *59:* 598–605, 1968.
21. Oster, I. I. The genetic Basis of X-ray Induced Somatic Damage. Proc. Second Australian Conf. Radiation Biol. 1958, 268–271, 1959.
22. Oster, I. I. Chemically Induced Life-shortening and Its Probable Genetic Basis. Science, *132:* 1497, 1960.
23. Ostertag, W. The Genetic Basis of Somatic Damage Produced by Radiation in Third Instar Larvae of *Drosophila melanogaster.* I. Death Before Maturity. Z. Vererbungslehre, *94:* 143–162, 1963.
24. Ostertag, W. Koffein-und Theophyllinmutagenese bei Zell-und Leukozytenkulturen des Menschen. Mutation Res., *3:* 249–267, 1966.
25. Ostertag, W. Chemische Mutagenese an Menschlichen Zellen in Kultur. Abhandl. Mainzer Akad. Wiss. Lit., Mainz, 1–124, 1966.
26. Ostertag, W., Duisberg, E., and Sturmann, M. The Mutagenic Activity of Caffeine in Man. Mutation Res., *2:* 293–296, 1965.
27. Ostertag, W., and Fromme, H. G. Morphologische und Chromosomale Veraenderungen an Leukozyten- und HeLa-Zellkulturen des Menschen und Chromosomenverlust bei *Drosphila melanogaster,* 9. Tagung der Deut. Gesellschaft Anthropol. Humangenetik 1965, Homo, 176–186, 1967.
28. Ostertag, W., and Greif, B. J. Die Erzeugung von Chromatidenbruechen durch Coffein in Leukocytenkulturen des Menschen. Humangenetik, *3:* 282–294, 1967.
29. Ostertag, W., and Haake, J. The Mutagenicity in *Drosophila melanogaster* of Caffeine and of other Compounds Which Produce Chromosome Breakage in Human Cells in Culture. Z. Vererbungslehre, *98:* 299–308, 1966.
30. Ostertag, W., and Kersten, W. The Action of Proflavin and Actinomycin D in Causing Chromatid Breakage in Human Cells, Exptl. Cell Res., *39:* 296–301, 1965.
31. Ostertag, W., and Muller, H. J. Genetic Basis of Somatic Damage Produced by Radiation. Science, *130:* 1422–1423, 1959.
32. Puck, T. T., and Steffen, J. Life Cycle Analysis of Mammalian Cells, I. A Method for Localizing Metabolic Events within the Life Cycle, and Its Application to the Action of Colcemide and Sublethal Doses of X-irradiation. Biophys. J., *3:* 379–397, 1963.
33. Rauth, A. M. Evidence for Dark-Reactivation of Ultraviolet Light Damage in Mouse L Cells. Radiation Res. *31:* 121–138, 1967.
34. Razavi, L. Single Stranded DNA in Lymphocyte Chromosomes. Nature, *215:* 928–931, 1967.
35. Somers, E. C., and Hsu, T. C. Chromosome Damage Induced by Hydroxylamine in Mammalian Cells. Proc. Natl. Acad. Sci. U.S., *48:* 937–943, 1962.
36. Ts'o, P. O. P. Physical Binding of Thymidine, Adenine, Caffeine, Steroids and Phenanthrene to DNA and Poly-A. Federation Proc., *22:* 583-1963.
37. Ts'o, P. O. P., Helmkamp, G. K., and Sander, C. Interaction of Nucleosides and Related Compounds with Nucleic Acids as indicated by the Change of Helix-coil Transition Temperature. Proc. Natl. Acad. Sci. U.S. *48:* 686–697.
38. Witkin, E. M. Modification of Mutagenesis Initiated by Ultraviolet Light through Posttreatment of Bacteria with Basic Dyes. J. Cellular Comp. Physiol., *58:* 135–144, 1961.
39. Wragg, J. B., Carr, J. V., and Ross, V. Inhibition of DNA Polymerase Activity by Caffeine in a Mammalian Cell Line. J. Cell Biol., *35:* 146A–147A, 1967.
40. Yanders, A. F., and Seaton, R. K. The Lack of Mutagenicity of Caffeine in *Drosophila.* Am. Naturalist, *46:* 277–280, 1962.
41. Zetterberg, G. The Mutagenic Affect of 8-ethoxycaffeine, Caffeine and Dimethylsulfate in the Ophiostoma Back-mutation Test. Hereditas, *46:* 279–311, 1959.

Fig. 1. HeLa cells untreated. × 18,600.
Fig. 2. HeLa cells. Treated with 1% caffeine. Cells start to round up. Beginning nuclear segmentation. × 18,000.
Fig. 3. HeLa cells. 1% caffeine. Expulsion of nuclear material
Fig. 4. HeLa cells. 1% caffeine. 12 hr. Nuclear fragmentation × 18,000.
Fig. 5. HeLa cell. 1% caffeine. 12 hr. Cell and nucleus completely fragmented × 20,000.
Fig. 6. Nuclear fragments as seen outside the cells are no longer incorporating thymidine-^{3}H. Normal amount of label inside the cells. × 6600.
Fig. 7. HeLa cells. 1% caffeine. DNA synthesis perinuclear (labeled with thymidine-^{3}H). × 18,400.
Fig. 8. HeLa cells, control. Label is found throughout the nucleus × 18,800.
Fig. 9. Caffeine-induced breaks in leukocyte cultures. Treatment (2 hr, 1% caffeine) during G-1 phase. (Reproduced from Ref. 28)
Fig. 10. Caffeine-induced breaks in leukocyte cultures. Treatment (2 hr, 1% caffeine) during late S-phase. (Reproduced from Ref. 28)

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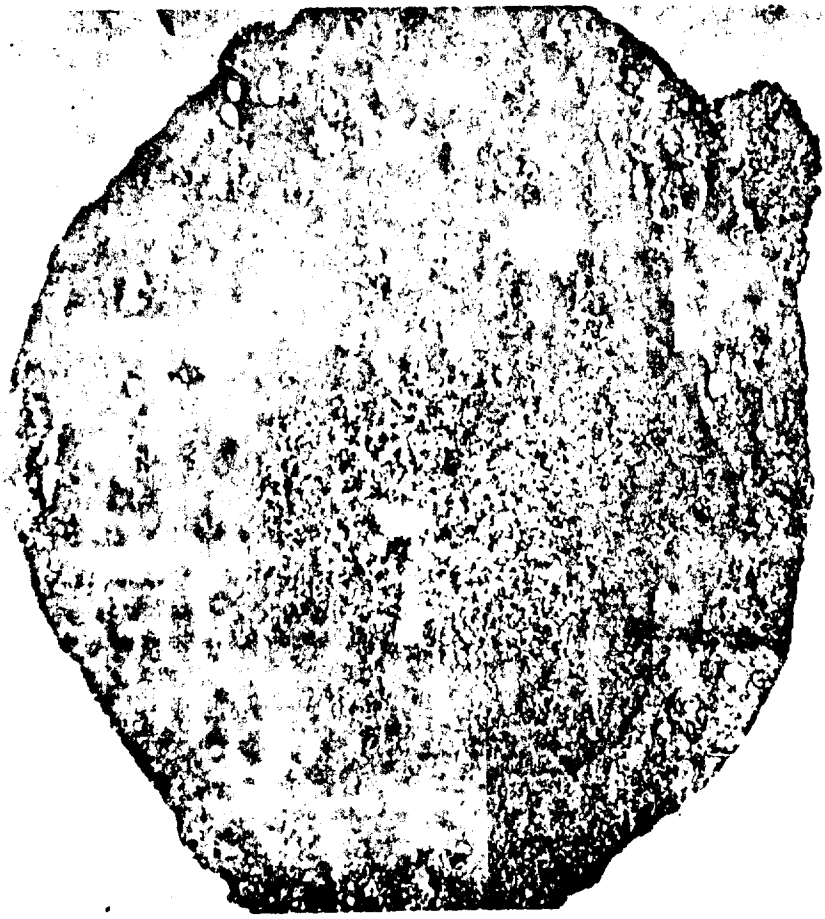

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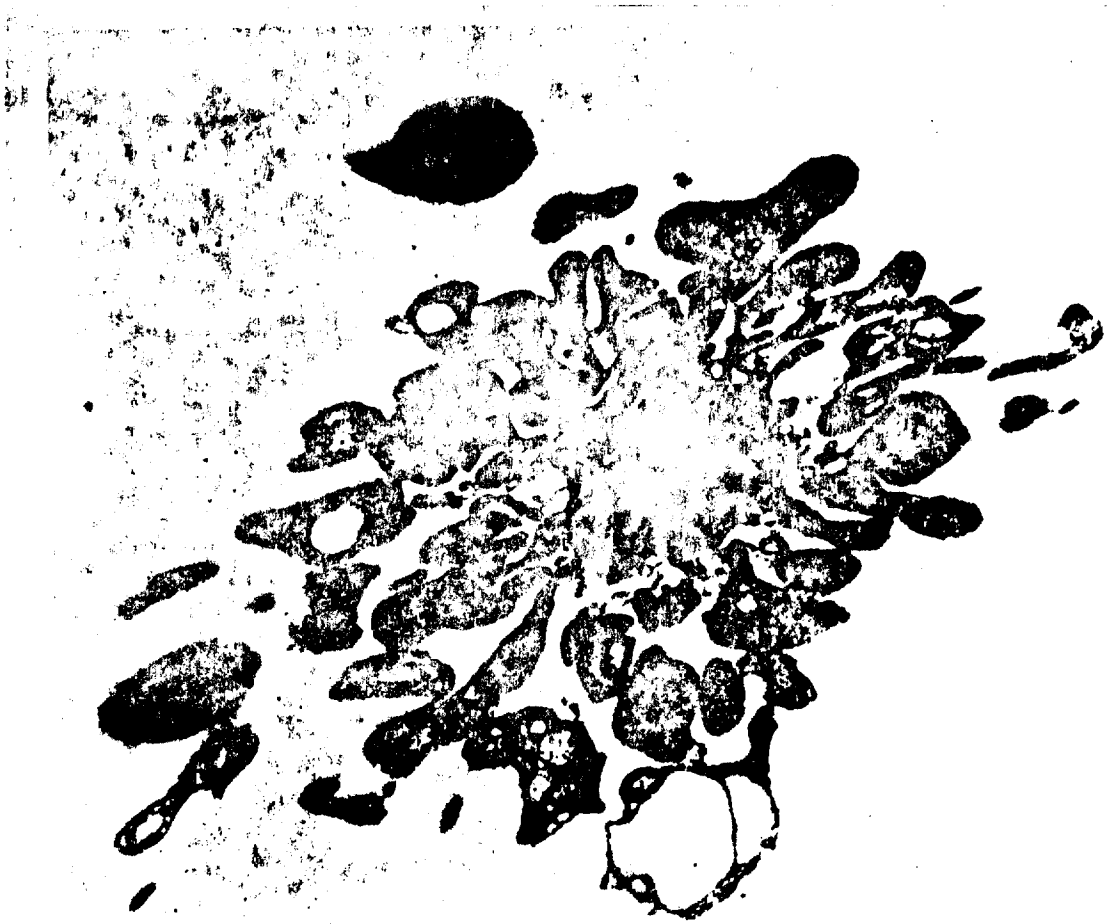

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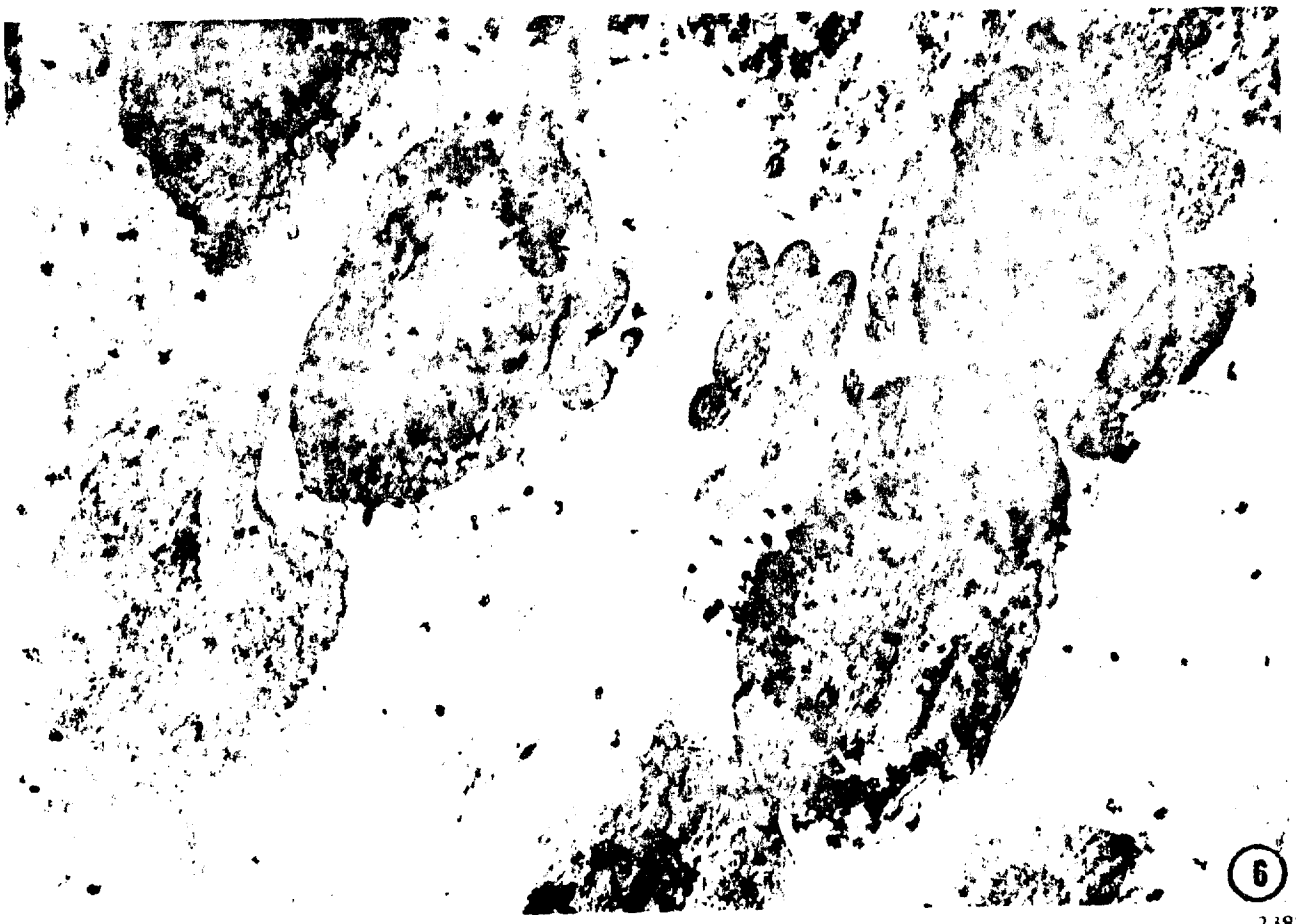

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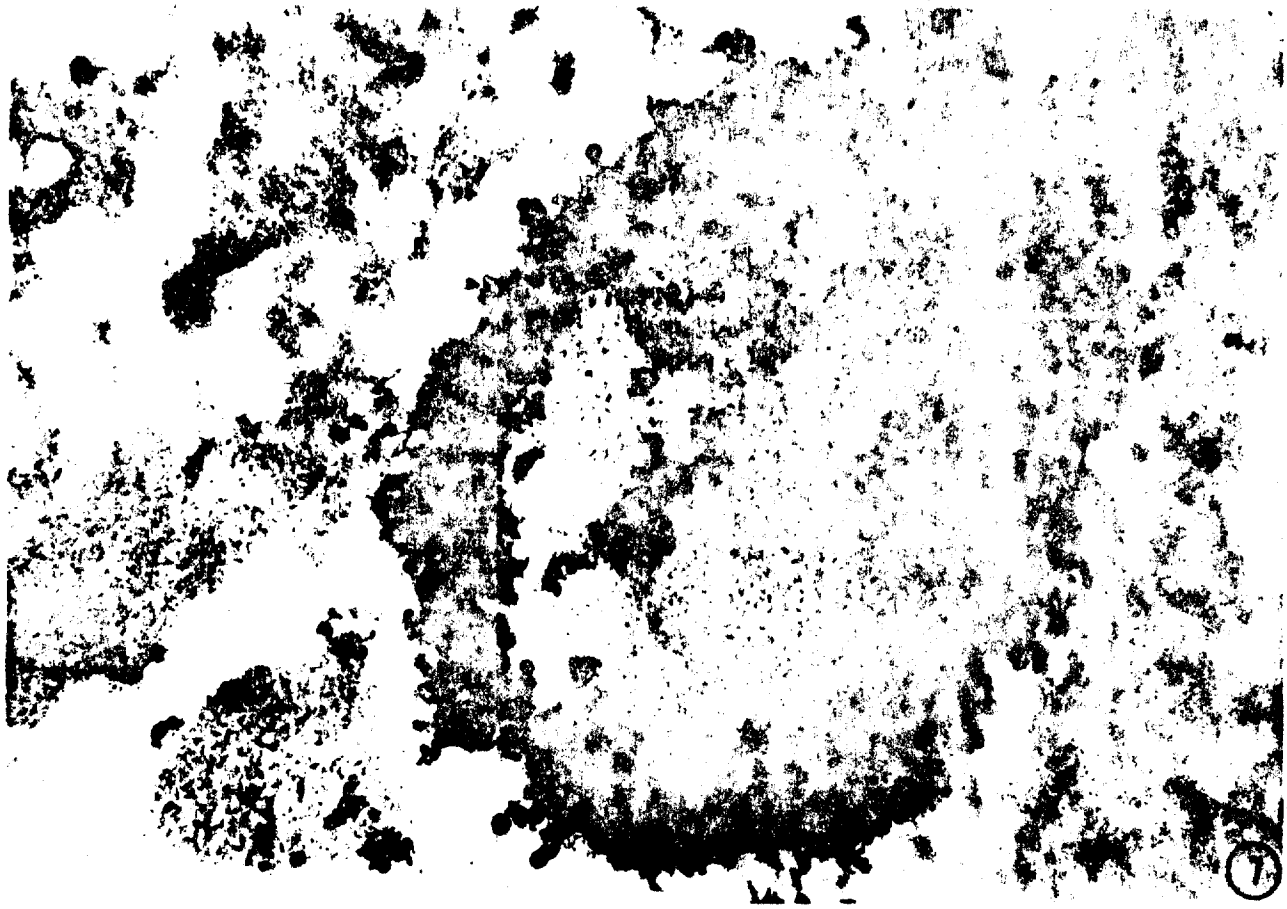

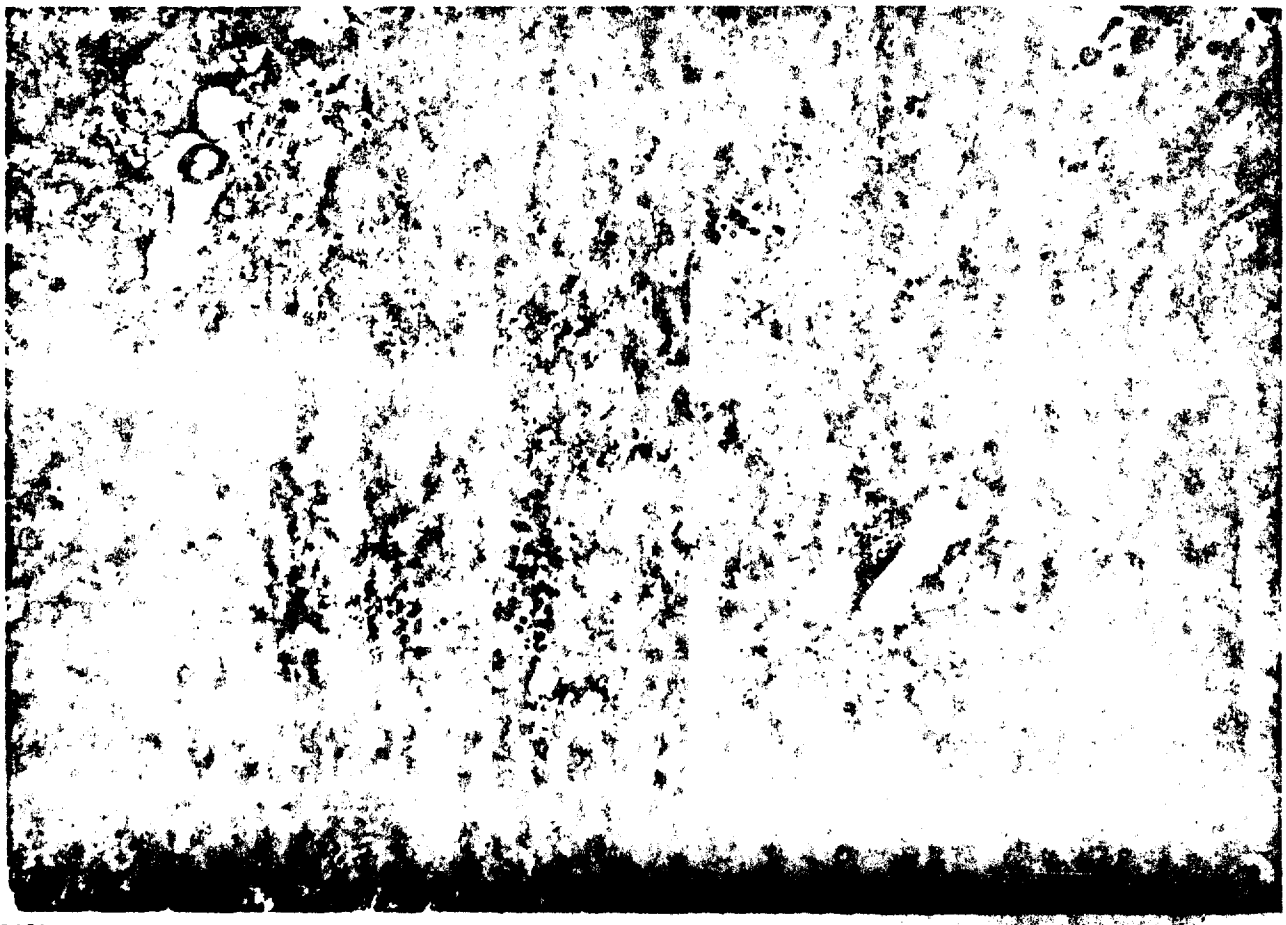

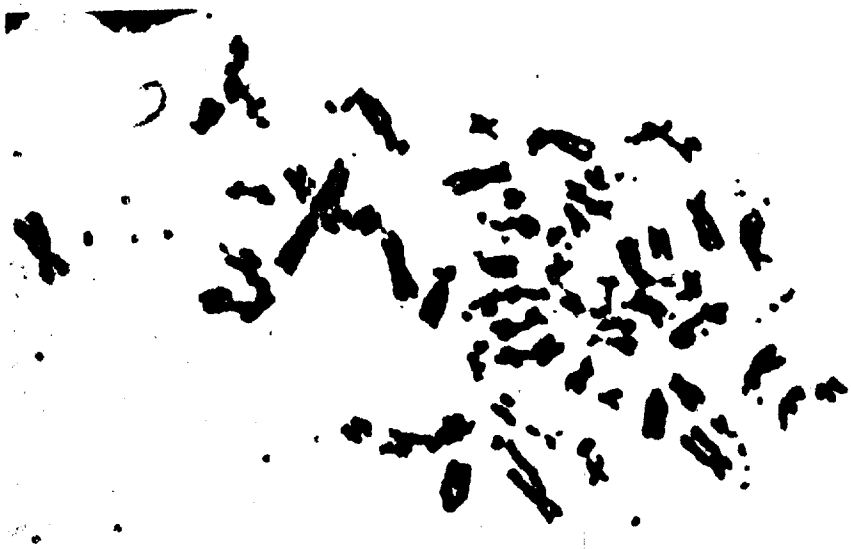

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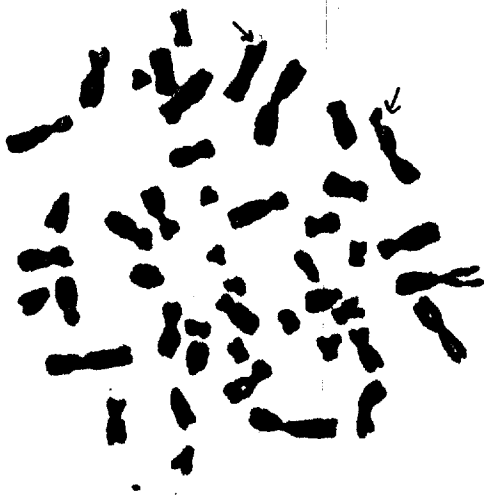

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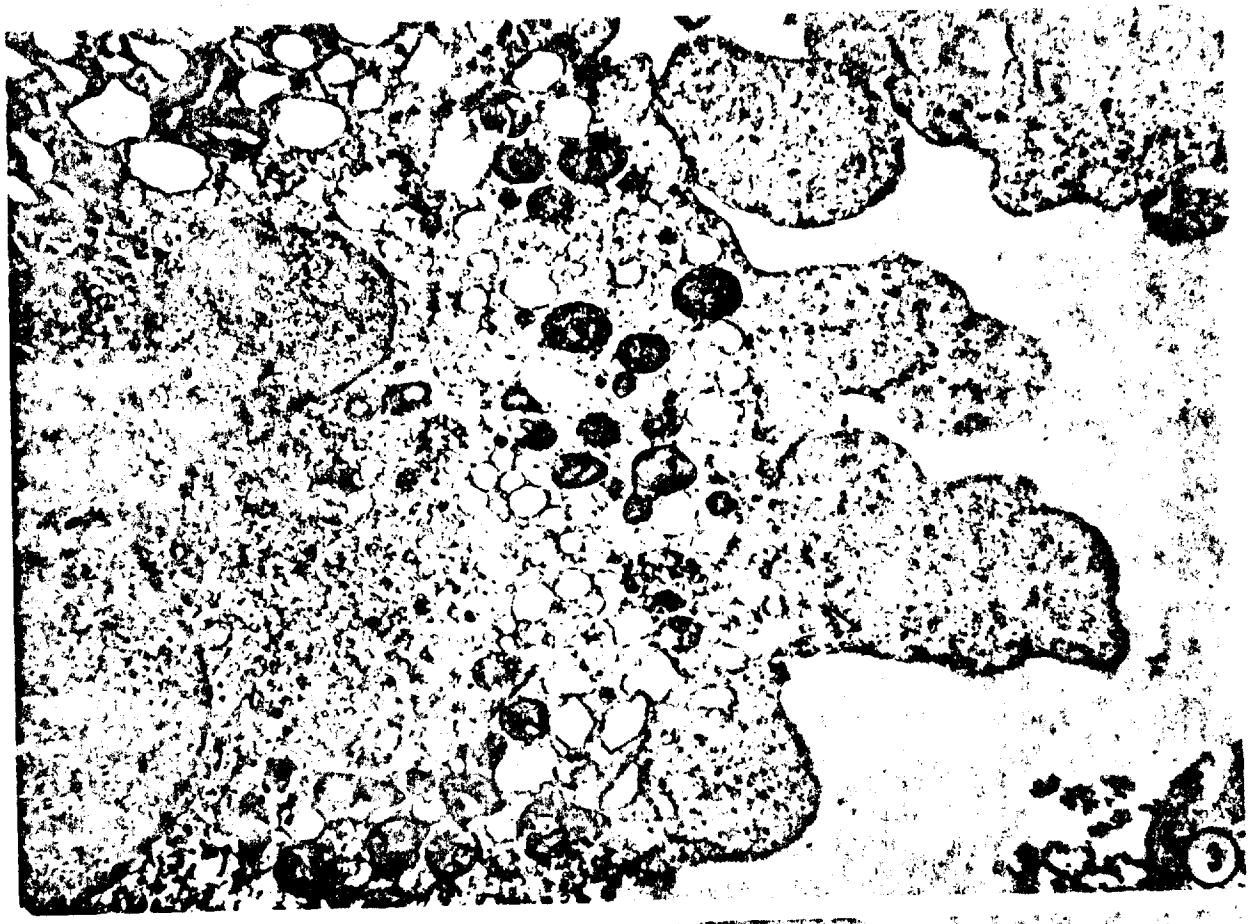

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The Effect of Coffee on the Function of the Sympatho-adrenomedullary System in Man

By

LENNART LEVI

Beverages containing methyl purines are consumed in very considerable quantities all over the world. This is especially true of coffee, the total annual consumption of which amounts to two million tons. The Swedes are among the biggest consumers, their annual consumption of roasted coffee being 9.7 kg per capita in 1964. Calculating with an average caffeine percentage of 1.3 (32), this corresponds to a mean annual ingestion of more than 120 g of pure caffeine (1-3-7-trimethylxanthine), i.e. a very substantial amount of a pharmacologically active compound. To this calculation must be added considerable amounts of other methyl purines, notably the theophylline of tea and theobromine of cocoa.

The effects of caffeine and coffee have been extensively studied as regards cardiovascular, respiratory, central nervous, and renal function. By contrast, very few studies have been devoted to the biochemical and endocrine effects of the compounds in question.

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Voznyuk (33) reports that 0.1 g of caffeine three times daily enhanced the uptake of I^{131} into the thyroid gland in patients with mild thyrotoxicosis.

Skebelskaja (29) reports that the effect of "stress" in reducing the ability of the rat thyroid to accumulate I^{131} after ACTH was less marked after administration of 50 mg of caffeine, possibly because of an intensified excretion of thyrotropic hormone from the hypophysis. Amigarova (2) found that caffeine depressed the production and stimulated the secretion of thyroid hormone. She also demonstrated that adrenaline had similar effects and proposed as a possible mechanism, an increased adrenaline liberation due to arousal of activity induced in the central nervous system by caffeine.

Abelson and Borcherds (1) report the appearance of a steroid-like substance in plasma after ingestion of coffee. Nishizawa and Eik-Nes (23) similarly report a caffeine-steroid complex in human urine, whereas Zelinsky and Patorzhinsky (34) were unable to pre-

sent contrary evidence concerning the influence of caffeine on the function of the adrenal cortex.

Lucarelli (22) reports that serotonin protects animals against the convulsive effects of large doses of caffeine.

Lenfeld et al. (19) found that caffeine and adrenaline inhibit serotonin oedema in a similar way, suggesting that the caffeine effect may be mediated by the catecholamines.

De Schaepdryver (7) reports that intravenous injection of caffeine markedly stimulates the secretion of adrenaline and, additionally, the secretion of noradrenaline as measured in supra-renal venous plasma in mongrel dogs.

However, no study has been made of the effects of coffee-consumption on the sympatho-adrenomedullary function in man as reflected in the urinary excretion of adrenaline and noradrenaline. This was accordingly the object of the present investigation.

Material and methods

The subjects were 9 healthy medical students (mean age 27, range [illegible]), 8 men and 1 woman. None of them were on a drug regimen. They were instructed to go to bed not later than midnight on the day before the experiment and without two glasses of tap water at bedtime. On the day of the experiment they emptied their bladders on awakening, drank two glasses of tap water, did not drink anything else or eat, and reported at the laboratory at 7.45 a.m. The experiment started at 8 a.m.

All details in the experimental setting were strictly standardized [illegible] instructions, stimuli, posture, [illegible] and fluid intake, [illegible] and urine collections during each [illegible] consecutive 2-hour period[illegible]

use of tobacco or any kind of drug was strictly forbidden (21).

During the 2nd 2-hour period the subjects were served 3 ordinary cups (300 ml) of sweetened black brand coffee, which chromatographic analysis showed to contain 75 mg caffeine per 100 ml of coffee brew. In addition, they were given two standard sandwiches with ham. This "coffee period" was preceded and followed by "control periods" with exactly the same procedure, except that the coffee was replaced by 300 ml of tap water, similarly sweetened. Urine samples were collected at the end of each period and analyzed fluorimetrically for adrenaline and noradrenaline by the method of von Euler and Lishajko (10). Urinary creatinine was estimated by the autoanalyzer method described in the Technicon Laboratory Manual.

There is a possibility that, for diurnal or other reasons, the catecholamine excretion may vary during the hours of the "coffee period" even when no coffee is ingested. An experiment from another series will accordingly be reported here to illustrate this factor. In this "control experiment", 8 young healthy medical students were allowed to sit comfortably during the same three 2-hour periods. The conditions were very similar to the present study, the only significant difference being that no coffee was served. Five of these control subjects were women and 3 men (mean age 24, age range 23—26). The sex and age differences between the "coffee" and control groups are very probably without any importance in this context (11).

Results

During the "coffee period", adrenaline increased by 80 % from 5.1 ± 0.6 ng/min to 9.[illegible] ng/min, followed by a fall in the second control period to 7.2 ± 0.9 ng/min. The difference in adrenaline excretion between the coffee period and the mean of the two control periods was statistically significant, $P < [illegible]$

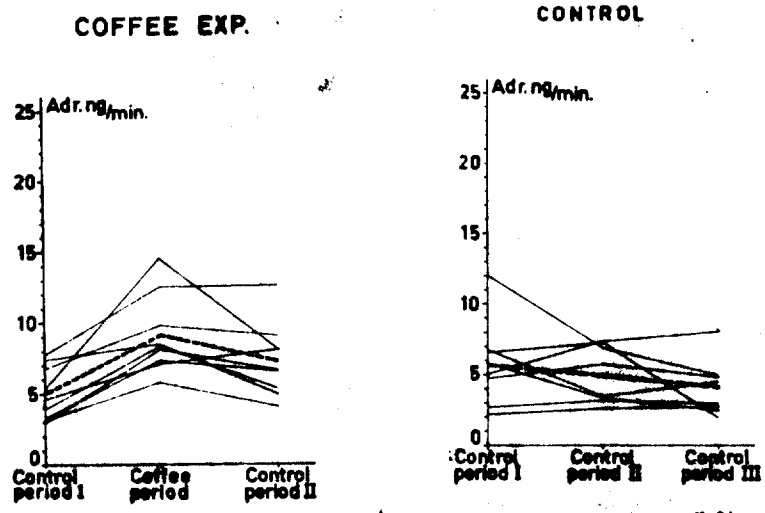

Fig. 1. Urinary excretion of adrenaline (ADR) during the coffee experiment (left) and under control conditions (right). Short-dash line indicates mean values.

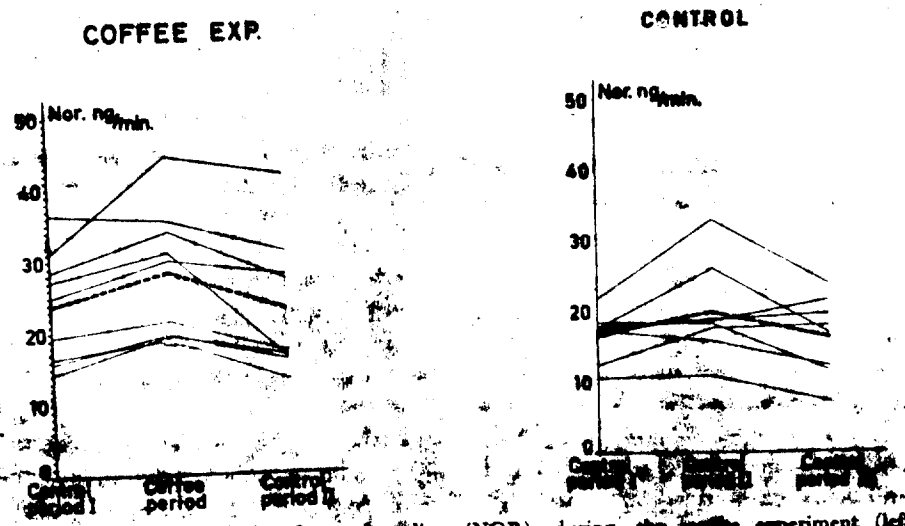

Fig. 2. Urinary excretion of noradrenaline (NOR) during the coffee experiment (left) and under control conditions (right). Short-dash line indicates mean values.

Noradrenaline excretion followed the same pattern, but the changes were less pronounced. During the coffee [illegible] subsequent fall reaching the level of the first control period. The difference between the coffee period and the mean [illegible] is statistically significant ($P < 0.01$).

Table I. The effects of coffee ingestion on some endocrine, renal and psychological variables

Variable	Period	Mean	S.E.M. ±
Adrenaline (ng/min)	Control I	[illegible]	0.6
	Coffee	9.1**	0.9
	Control II	7.2	0.9
Noradrenaline (ng/min)	Control I	23.9	2.6
	Coffee	28.4***	3.0
	Control II	28.2	3.2
Urine volume (ml/min)	Control I	1.37	0.4
	Coffee	1.32**	0.2
	Control II	1.47	0.2
Specific gravity	Control I	1.016	0.003
	Coffee	1.019**	0.002
	Control II	1.016	0.002
Urinary [illegible]	Control I	1.22	0.10
	Coffee	1.25**	0.07
	Control II	1.25	0.06
Feelings of well-being	Control I	6.2	0.5
	Coffee	7.6**	0.6
Questionnaire scores	Control II	6.1	0.7
Feelings of indolence	Control I	5.3	0.7
	Coffee	5.7*	0.7
Questionnaire scores	Control II	[illegible]	[illegible]

*, **, and *** indicate that [illegible] 0.05, 0.01, and 0.001, respectively, [illegible] difference [illegible] 2 control periods; ns [illegible] not significant.

Urine volume was not significantly different during the coffee period in relation to the two control periods, nor was specific gravity or the urinary [illegible] excretion (table I).

As to subjective feelings reported in the questionnaires, [illegible] arousal and well-being [illegible] increase (table I). No [illegible] attributable to the [illegible]

Discussion

A comparison of the catecholamine data mentioned above with those of the control experiment demonstrates (figs. 1 and 2) that the adrenaline changes found to occur during the coffee period were in all probability primarily due to the ingestion of coffee. An additional factor, though probably of much less importance, may be that the subjects of course knew from previous experience the general properties of the fluid they had to ingest. This knowledge might have affected their subjective reactions and also, secondarily, their objective reactions (12, 13).

The results reported above thus demonstrate that the adrenaline excretion as estimated fluorimetrically rises considerably after the ingestion of what is usually considered to be a moderate quantity of coffee, the rise being of the same magnitude as that occurring in industrial or office stress situations or during emotionally charged film programmes (20, 21). The question arises whether this really reflects an increased production and/or release into the blood stream of catecholamines. The possibility exists that caffeine interferes with the assay method, although this is unlikely because of the chemical differences between the compounds in question. In order to make sure, caffeine (25 mg) was added to three 25 ml urine samples. These samples and [illegible] without any added [illegible] found that the compound added did not influence the fluorescence readings. In [illegible]

been adjusted to the alkaline side — a procedure which is known to make the free catecholamines disappear from the solution. There is *a priori* no reason to expect caffeine and its metabolites to react in the same way. Thus, if after such treatment some fluorescence is left in the urines from the coffee period, it would presumably be attributed to caffeine or its metabolites. However, all fluorescence disappeared. This supports the view that the fluorescence was probably not due to the compounds in question, but of course does not prove it (6).

It may further be argued that the adrenaline increase merely reflects a change in renal function. However, this is highly unlikely, as the urine volume, specific gravity, and creatinine excretion remained substantially unchanged during the coffee period. In addition, it has repeatedly been shown that increases in plasma catecholamines are readily reflected as an increased urinary catecholamine excretion (9).

The present interpretation finds further support in the increased catecholamine secretion into adrenal venous blood following the administration of caffeine (7).

A controversial question is whether the psychological and physiological reactions usually induced by coffee are due exclusively to its caffeine content or to other coffee constituents as well (5). Compounds such as [illegible], acetone, furfuran, ammonia, methyl[illegible], trimethylamine, fum[illegible] acid, resorcinol, hydro[illegible], and [illegible] [illegible]

feinated instant coffee containing roughly one-fortieth of the amount of caffeine found in regular coffee (32) lacks the typical coffee effects (27). The changes induced in flicker-fusion tests by caffeine, caffeine-containing regular coffee as well as by subcutaneous injection of small amounts of adrenaline, but not by decaffeinated coffee (30), also suggest that coffee constituents other than caffeine are not of major importance as regards the effects induced by coffee. So do the studies by Seyffert (28) and Kligge (16), in which no obvious effects whatsoever could be demonstrated in subjects after drinking decaffeinated coffee. Bellet et al. (4) found that 15 normal subjects who were given regular coffee showed an increase in plasma free fatty acids; in 9 normal subjects who were given decaffeinated coffee, there was no significant change from the baseline. In addition, preliminary results from a recent study (18) indicate that oral administration of pure caffeine (200 mg) also produces an increased adrenaline excretion.

In view of the catecholamine-releasing effects of other stimulating drugs, such as amphetamine (3) and tobacco (31), it is *a priori* not astonishing that coffee, too, induces an increased catecholamine release. It is noteworthy that the increased output of 5-hydroxyindolacetic acid induced by caffeine is also found with metamphetamine (Pervitin) and fenmetralinhydrochloride (Preludin), the increase in urinary excretion of 5-HIAA being parallel to the stimulating action of the drug (8).

Amphetamine, methylphenidate (Ritalin) and adrenaline have in fact been

grouped together with caffeine on the [illegible] of their effects on the electrical [illegible] of the brain (15).

The present findings have two obvious [illegible]. The first concerns coffee [illegible] of error in experiments and [illegible] procedures centering on the [illegible] catecholamine excretion. Ad[illegible], the diagnosis of phaeochromo[illegible] is unlikely to be confused by the [illegible] adrenaline rise, but in all [illegible] there is good reason [illegible] coffee ingested for at least [illegible] hours prior to the experiment. The [illegible] of caffeine has been shown [illegible] be approximately 5 hours (26). [illegible] second implication is a clinical [illegible]. Is it reasonable to suppose that large [illegible] of coffee repeatedly ingested over [illegible] periods of life may be detrimental [illegible] health, either in themselves or in combination with other [illegible]? Although there are [illegible] answers, it is tempting to speculate [illegible].

Raab (23) has pointed out the possibly detrimental effects of prolonged sympathotonia on myocardial vulnerability. Paul et al. (24) have demonstrated a positive correlation between coffee [illegible] and the incidence of coronary heart disease. It may also be noted that Krohina (17) [illegible] caffeine to dogs, provoked a focal interstitial myocarditis [illegible] injuries of the intramural nervous system in the heart. Of course, [illegible] experiment in no way reproduced what may happen during physiological normal life conditions, i.e. when drinking [illegible] [illegible] remains [illegible]

can contribute to a sympathotonia which in the long run may have a pathogenic effect on organ function.

Reporting some cases of caffeine poisoning, Jokela and Vartiainen (14) comment that single doses in excess of about 1 g bring on a marked state of agitation, disturbances in heart function, pains of angina pectoris type, gastro-intestinal symptoms, acute diuresis, tremor of the muscles, and even convulsions. However, people react in markedly different ways to caffeine, some being able to take considerable doses without any alarming symptoms, probably because of habitual use of caffeine-containing beverages. The reactions to caffeine are also said to depend upon the functional state of the autonomic nervous system (16). Thus, the question of potentially pathogenic effects of coffee is a complicated one and deserves further study.

Summary

In 9 young healthy medical students, the ingestion of 3 cups (300 ml) of black brand coffee caused a significant increase in urinary adrenaline excretion. The discussion centres on whether this increase reflects an increased production and/or release of adrenaline from the adrenal medulla, and if so, what is the clinical significance of a repeated stimulation of this kind. It is concluded that there is good reason to study further the biochemical and endocrine effects of this pharmacologically potent [illegible]

References

1. ABELSON, D. & BORCHERDS, D.: Substance appearing in steroid-containing plasma extracts after ingestion of tea or coffee. Nature (Lond.) *179:* 1135, 1957.
2. AMIGAROVA, M. G.: The effect of caffeine and barbamyl on the secretory cycle of the thyroid gland. Bull. exp. Biol. Med. *54:* 54, 1962.
3. AXELROD, J.: Biochemical factors in the formation of active and toxic metabolic products. Drugs and enzymes. Proceedings of the 2nd Int. Pharmacol. Meeting in Prague 1963. p. 309. Praha 1965.
4. BELLET, S., ASPE, J., KERSHBAUM, A. & ZANUTTINI, D.: Effect of caffeine on free fatty acids. Circulation Suppl. *3:* 45, 1964.
5. CZOK, G.: Untersuchungen über die Wirkung von Kaffee. Steinkopf Verlag, Darmstadt 1966.
6. DASTUR, D. K., MANN, J. D. & POLLIN, W.: Hippuric acid excretion, coffee, and schizophrenia. Arch. gen. Psychiat. *9:* 79, 1963.
7. DE SCHAEPDRYVER, A. F.: Physio-pharmacological effects on suprarenal secretion of adrenaline and noradrenaline in dogs. Arch. int. Pharmacodyn. *119:* 517, 1959.
8. DEOKWITZ, R. & SIEROSLAWSKI, H.: Erhöhung der 5-Hydroxyindolessigsäure (HIES) Ausscheidung im Harn nach Coffein, Pervitin und Preludin. Klin. Wschr. *41:* 902, 1963.
9. ELMADJIAN, F., HOPE, J. M. & LAMSON, E. T.: Excretion of epinephrine and norepinephrine in various emotional states. J. clin. Endocr. *17:* 608, 1957.
10. VON EULER, U. S. & LISHAJKO, F.: Improved technique for the fluorimetric estimation of catecholamines. Acta physiol. scand. *51:* 348, 1961.
11. VON EULER, U. S.: Noradrenaline. Thomas, Springfield, 1956.
12. FRANKENHAEUSER, M., JARPE, G., SVAN, H. & WIRANOSJÖ, B.: Psychophysiological reactions to two different [illegible] treatments. Scand. J. Psychol. [illegible] 74, 1964.
13. HILL, [illegible]
barbital. Arch. Neurol. Psychiat. (Chic.) *77:* 28, 1957.
14. JOKELA, S. & VARTIAINEN, A.: Caffeine poisoning. Acta Pharmacol. (Kbh.) *15:* 331, 1959.
15. JOUVET, M., BENOIT, O., MARSALLON, A. & COURJON, J.: Action de la caféine sur l'activité électrique cérébrale. C. R. Soc. Biol. (Paris) *151:* 1542, 1957.
16. KLIGGE, H.: Die vegetative Dystonie, die Hypertonie und der Bohnenkaffee. Med. klin. *57:* 1103, 1962.
17. KROHINA, E. M.: Histopathology of the nervous elements of the heart and aorta intravenous administration of adrenaline and caffeine to dogs. Bull. exp. Biol. Med. *52:* 112, 1961.
18. LEANDERSON, R. & LEVI, L.: Pharmacological modification of biochemical and behavioural reactions during control conditions and during conditions of experimentally induced emotional stress. Paper pres. Int. Symposium on Antidepressant Drugs, Milan 1966. Excerpta med. ICS *122:* 75, 1967.
19. LENFELD, J., SLADKOVÁ, O. & GRUNDMAN, M.: On the mechanism of inhibition of inflammatory edema by caffeine with regard to serotonin. Čas. Lék. čes. *102:* 554, 1963.
20. LEVI, L.: The urinary output of adrenaline and noradrenaline during experimentally induced emotional stress in clinically different groups. Acta psychother. (Basel) *11:* 218, 1963.
21. LEVI, L.: The urinary output of adrenaline and noradrenaline during pleasant and unpleasant emotional states. Psychosom. Med. *27:* 80, 1965.
22. LUCARELLI, A. F.: 5-Idrossitriptamina e convulsioni sperimentali da caffeina nel topolino. Boll. chim. farm. *97:* 16, 1958.
23. NISHIZAWA, E. E. & EIK-NES, K. B.: The presence of a possible caffeine steroid complex in human urine. J. Chromatogr. *16:* 493, 1963.
24. PAUL, O., LEPPER, M. H., PHELAN, W. H., DUPERTUIS, G. W., MACMILLAN, A., MCKEAN, H. & PARK, H.: A longitudinal study of coronary heart disease. Circulation *28:* 20, 1963.

25. RAAB, W.: The nonvascular metabolic myocardial vulnerability factor in "Coronary heart disease". Amer. Heart J. *66:* 685, 1963.

26. SANT'AMBROGIO, G., MOGNONI, P. & VENTRELLA, L.: Plasma levels of caffeine after oral intramuscular and intravenous administration. Arch. Int. Pharmacodyn. *150:* 259, 1964.

27. SCHNEIDER, W.: Die Wirkung von coffeinhaltigem Kaffee auf die Flimmerverschmelzungsfrequenz. Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak. *227:* 393, 1956.

28. SEYFFERT, H.-M.: Physiologische and psychologische Wirkungen von Kaffee und Coffein. Arzneimittel-Forsch. *4:* 207, 1954.

29. SKEBELSKAJA, J. B.: The effect of caffeine on the reaction of the thyroid gland to "stress". Probl. Endokr. Gormonoter. *6:* 7, 1960.

30. WACHHOLDER, K. & SCHNEIDER, W.: Die Änderung der Flimmerverschmelzungsgrenze auf coffeinhaltigen Kaffee und deren Abhängigkeit von der vegetativen Ausgangslage. Klin. Wschr. *34:* 276, 1956.

31. WESTFALL, T. C.: Tobacco alkaloids and the release of catecholamines. In: Tobacco alkaloids and related compounds. Ed. U. S. von Euler. p. 179. Pergamon Press, Oxford 1964.

32. WOLMAN, W.: Instant and decaffeinated coffee. J. Amer. med. Ass. *159:* 250, 1955.

33. VOZNYUK, B. I.: The importance of caffeine loading in the diagnosis of hyperfunction of the thyroid gland. Med. Radiol. (Moskva) *6:* 23, 1961.

34. ZELINSKY, S. P. & PATORZHINSKY, A. M.: Effect of aminasine and caffeine on the function of the adrenal cortex in schizophrenia patients. Fiziol. Zh. (Kiev) *9:* 651, 1963.

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DIFFERENTIAL EFFECTS OF COFFEE ON SPEED AND POWER TESTS*

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A. PROBLEM

Considerable information has been gathered concerning the effects of coffee on psychological test performance. Positive as well as negative results have been reported [see Eichler (4), Gilliland and Nelson (6), and De Boor (2, p. 126-130)]. Careful study of these reports suggests that positive effects occur most often in performance on tests requiring speed. Until now the speed-power characteristics of the tests applied have not been controlled when determining the influence of coffee on the test performance. The following study was carried out to examine the question of whether the effect of coffee on performance of speed tests is different from its effect on performance of power tests.

B. METHOD

1. *Subjects*

Two randomly selected groups of *S*s, an experimental group and a control group, were used. Each group consisted of 50 male and female students from the Liberal Arts and Sciences Departments of the University of Marburg. The students ranged in age from 20 to 32 years.

2. *Tests*

Each *S* was given two standardized tests in an individual session: (*a*) the Intelligenzstrukturtest (IST) by Amthauer (1), a battery of nine more or less speeded power tests, and (*b*) the Konzentrationsleistungstest (KLT) by Düker and Lienert (3), a typical speed test consisting of 250 relatively easy arithmetical items of the form $8 - 3 + 7 = ?$

* Accepted by Joseph Zubin of the Editorial Board, received in the Editorial Office, Provincetown, Massachusetts, on April 29, 1966, and published immediately at 35 New Street, Worcester, Massachusetts. Copyright by The Journal Press.

[1] The experiment was performed by L. Kangieser, junior student at the Psychological Department of the University of Marburg (Germany), under supervision of the senior author.

3. *Treatment*

Fifteen minutes prior to testing, *S*s of the experimental group were given a cup of strong coffee (Nescafé) containing .3 grams of caffeine. *S*s of the control group did not receive any drink.

C. Hypotheses and Predictions

It was hypothesized that speed of test performance would be increased by caffeine, whereas power of performance would remain unchanged or be decreased. The theoretical implication was that the dynamic factors of intelligence should be positively influenced; the cognitive factors on the other hand should be influenced either not at all or perhaps negatively. From this hypothesis the following predictions were derived:

1. Performance in the typical speed test (KLT) will increase under coffee.
2. Performance in the more or less speeded power tests of the IST battery will increase or decrease depending upon their degree of speeding.

The degree of speeding of a single test of the IST battery was to be measured by its correlation with the typical speed test KLT.

D. Results

With respect to the first prediction, the standard score means and variances ($\mu = \sigma^2 = 100$) of the speed test (KLT) presented in Table 1 were obtained. These data indicate that the coffee group performed better than the control group. The difference between means is significant at the 1 per cent level of confidence. Thus Prediction 1 is confirmed.

Results concerning the means and variances of the standard scores of the partly speeded IST subtests ($\mu = \sigma^2 = 100$) are given in Table 2. According to Table 2, performance in three of the nine subtests increased (numerical reasoning, RA, inductive reasoning, ZR, and spatial reasoning, FA); whereas performance in two other subtests (general information, SE, and verbal comprehension, GE) decreased.

To decide whether the subtests positively influenced by coffee require more speed than the subtests not positively influenced, product-moment-correlations

TABLE 1
Means and Variances for Both Groups on the Speed Test

KLT	Experimental group	Control group	*N*
μ	116.1	102.3	50
σ^2	122.9	106.9	50

TABLE 2
MEAN STANDARD SCORES AND VARIANCES FOR BOTH GROUPS ON THE NINE IST SUBTESTS

IST subtest	Experimental group ($N = 50$) μ	σ^2	Control group ($N = 50$) μ	σ^2
SE = General information	108.0	41.3	116.6	38.6
WA = Verbal classification	110.2	65.1	108.9	55.1
AN = Semantic analogies	111.0	54.3	112.3	47.1
GE = Verbal comprehensions	110.0	40.4	115.0	80.8
ME = Associate memory	107.9	58.3	108.8	51.9
RA = Numerical reasoning	108.9	107.1	107.4	102.4
ZR = Inductive reasoning	112.5	85.3	110.2	150.1
FA = Spatial relations	110.5	55.1	106.8	134.6
WÜ = Spatial orientation	107.8	117.4	106.7	114.3

between the KLT and each of the nine IST subtests were calculated and tested against the null hypothesis according to Table 3. Two of the three tests favored by coffee were found to be positively correlated with the typical speed tests, while the correlations of those tests not positively influenced did not differ significantly from zero. If all of the nine subtests are considered, a high positive rank correlation of rho = .82 was found between the speed criterion for the subtests and gains in performance under coffee. Thus, Prediction 2 must be also accepted as true.

E. DISCUSSION

The fact that only speeded tests were favored by coffee may be explained in terms of Thurstone's multiple factor theory of intelligence (10). The speed factor or the speed factors—at least one of which has been found in factorial studies [see Eysenck (5, p. 152) and Lord (9)]—should be of increased importance under coffee. The positive or negative effects of coffee upon particular tests may be the result of gains and losses, respective of this speed factor.

As yet unmentioned is the partial result that the variance of performance on some of the IST subtests (GE, ZR, and FA) was significantly smaller for the coffee group than for the control group (see Table 2). Obviously, coffee tends to reduce individual differences in specified intellectual abilities, as measured by the individual IST subtests. In terms of the multiple factor theory of intelligence, the effect may be explained tentatively by supposing different factorial test patterns in high and low scoring *S*s, a phenomenon that has been shown to exist [see Lienert (7)]. If, for example, the inductive reasoning test (ZR), given to a group of low-scoring *S*s, gains more in speed factor loading under coffee than the same test given to a group of high-

TABLE 3

SUBTEST INTERCORRELATIONS (PEARSONIAN r's) AND MEAN GAIN (IN STANDARD SCORE UNITS) UNDER COFFEE

IST subtest	SE	WA	AN	GE	ME	RA	ZR	FA	WÜ	N
Average correlation (r) with the KLT from both groups	.17	.22	—.04	—.16	.23	.45	.39	.29	.24	2 × 50
Mean gain under coffee	—3.6	1.3	—0.7	—5.0	—0.9	1.5	2.4	3.7	1.1	50

scoring *S*s, test performance of the low-scoring *S*s will increase more than test performance of the high-scoring *S*s, thus reducing variance of test performance. It is not established that the variance-reducing effect of coffee has anything to do with a hypothesized differential speed factor pattern. The problem should be examined empirically by separately factorizing high- and low-scoring groups of *S*s with and without administration of coffee.

Also open to question is the validity of our speed criterion. The correlations between the KLT and the IST subtests may not be due exclusively to common variance in the speed factor, but also to the common variance in a numerical factor. The latter may have unduly increased the correlation between the arithmetical KLT and the numerical tests ZR and RA. However, this objection may be rejected by considering a further speed criterion defined by Lienert and Ebel (8) and calculated for each of the nine IST subtests. With regard to this criterion, the two numerical tests (RA and ZR) were shown to be highly speeded just as with the original criterion. Rank correlation between the two criteria was found to be .72.

Finally, it should be stressed that the present investigation was not planned or performed to test the effects of caffeine nor—strictly speaking—to test the effects of coffee, but to test the effects of drinking coffee on performance in speed and power tests. Therefore the criticism that a placebo medication was not given to the *S*s of the control group is not relevant. It is clear that the intake of coffee produced some uncontrolled sets, and therefore it cannot be stated that the changes in test performance, observed in this experiment, are due solely to the effect of coffee. They will be at least partly due to the unlike motivational level of the two groups compared. So our conclusions are not far from being practically significant.

F. Summary

To test the hypothesis of differential effects of coffee on speed and power tests, two groups of 50 *S*s each were given 10 standardized tests, one of which was a typical speed test and the other nine of which were partly speeded power tests. Before testing, the *S*s of the experimental group were given a cup of Nescafé containing .3 grams of caffeine, while the *S*s of the control group did not receive any drink.

As predicted from prior empirical evidence and from theoretical considerations, mean performances of the speed tests and the mostly speeded power tests were increased significantly for the coffee group, whereas mean per-

formance in the less or nonspeeded power tests was either not affected or reduced.

Results were interpreted in terms of a multiple-factor theory of intelligence by changes in speed factor loading of the tests under caffeine.

References

1. Amthauer, R. Der Intelligenzstrukturtest (IST) (2nd ed.). Göttingen: Hogrefe, 1955.
2. De Boor, W. Pharmakopsychologie und Psychopathologie. Berlin: Springer, 1956.
3. Düker, H., & Lienert, G. A. Konzentrationsleistungstest (KLT). Göttingen: Hogrefe, 1959.
4. Eichler, O. Kaffee und Coffein. Berlin: Springer, 1938.
5. Eysenck, H. J., *et al.* Dimensions of Personality. London: Routledge, 1947.
6. Gilliland, A. R., & Nelson, D. The effects of coffee on certain mental and physiological functions. *J. Gen. Psychol.*, 1939, **21**, 339-348.
7. Lienert, G. A. Die Faktorenstruktur der Intelligenz als Funktion des Intelligenzniveaus. Bericht über den 22. Kongress der Deutschen Gesellschaft für Psychologie in Heidelberg, 1959. Göttingen: Hogrefe, 1960.
8. Lienert, G. A., & Ebel, O. Ein Index zur numerischen Bestimmung der Niveau-Eigenschaft eines psychologischen Tests. *Metrika*, 1960, **3**, 117-127.
9. Lord, F. M. A study of speed factors in tests and academic grades. *Psychometrika*, 1956, **21**, 31-50.
10. Thurstone, L. L. The Vectors of Mind. Chicago: Univ. Chicago Press, 1944.
11. ———. Multiple Factor Analysis. Chicago: Univ. Chicago Press, 1947.

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COFFEE AND SERUM-LIPIDS IN CORONARY HEART-DISEASE

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WE found no significant correlations between various nutrients and serum-cholesterol levels in healthy Canadians and patients with coronary heart-disease (Little et al. 1965). The single exception was a positive correlation between daily cups of coffee and serum-cholesterol in the coronary group ($r=0{\cdot}31$; $P<0{\cdot}01$). Paul et al. (1963) reported a correlation between coffee and the later development of coronary heart-disease ($P<0{\cdot}025$). We have extended our inquiry by correlating coffee with a number of serum-lipid and serum-lipoprotein fractions in patients with coronary heart-disease and in controls.

Patients and Methods

The selection and description of the patients and controls have been reported previously (Shanoff et al. 1961, Little and Shanoff 1963, Shanoff and Little 1964, Little et al. 1965). Briefly the coronary patients had survived a myocardial infarction for at least three months and were free of any other significant disease. There were about 25 men in each decade from the fourth to eighth decades, inclusive. Healthy men from the same population were matched by age as controls. All subjects were ambulatory at home and none was receiving a special diet.

A list of all ingested foods and fluids, except water, was recorded by the participants after each meal for seven consecutive days. Measured amounts—such as cups of coffee, glasses of milk, teaspoons of sugar, servings of potato—were noted but not weighed. The number of subjects who did not provide a satisfactory record was similar in each decade in both groups; thus no bias was introduced by their exclusion. The identity and diagnosis of the participants were unknown to the dietitian who analysed the records with food tables (Bowes and Church 1951, *Table of Food Values* 1951).

Fasting total, free, and esterified serum-cholesterols were estimated by the method of Sperry and Webb (1950), serum-phospholipid by the method of Zilversmit and Davis (1950),

TABLE I—CORRELATION COEFFICIENT OF DAILY CUPS OF COFFEE AND TEA WITH SERUM LIPID AND LIPOPROTEIN LEVELS IN CORONARY AND CONTROL SUBJECTS

Serum-fraction	Control (D.F. 82)		Coronary (D.F. 84)		Combined control and coronary (D.F. 168)	
	Coffee	Tea	Coffee	Tea	Coffee	Tea
Total cholesterol ..	−0·008	0·050	0·312†	−0·280†	0·162*	−0·138
Ester cholesterol ..	0·003	0·025	0·293†	−0·275*	0·159*	−0·147
Free cholesterol ..	−0·029	0·102	0·320†	−0·236*	0·163*	−0·097
Phospholipid ..	0·015	−0·121	0·332†	−0·226*	0·183*	−0·185*
Standard serum-fraction:						
0–12 lipoprotein	−0·052	0·122	0·312†	−0·204	0·169*	−0·090
12–20 lipoprotein	0·072	0·054	0·254*	−0·139	0·174*	−0·055
20–100 lipoprotein	0·099	0·067	0·230*	−0·165	0·172*	−0·069
100–400 lipoprotein	0·093	−0·027	0·370‡	−0·254*	0·263‡	−0·166*
0–20 lipoprotein	−0·015	0·113	0·332†	−0·210	0·188*	−0·090
20–400 lipoprotein	0·109	0·032	0·320†	−0·224*	0·229†	−0·121

Probability level for r: $<0{\cdot}05$ (*), $<0{\cdot}01$ (†), $<0{\cdot}001$ (‡).
D.F. = Degrees of freedom.

and the serum-lipoproteins by the ultracentrifuge method of DeLalla and Gofman (1954).

Results

The daily amounts of coffee, tea, and various nutrients ingested were given in detail previously (Little et al. 1965). There were no important differences between the control and coronary groups with regard to the intake of calories, coffee, tea, total carbohydrate, sucrose, total fat, milk-fat, and eggs.

The correlation coefficients of coffee and tea with the serum-lipids are shown in table I. In the control group none of the correlations is significantly different from zero. In the coronary group coffee shows a consistently positive and statistically significant correlation with each serum-lipid and serum-lipoprotein fraction. Tea, however, has consistently negative correlations, but not all are significant. Combining the control and coronary groups gives results comparable to those of the coronary patients alone, but at a lower level of significance.

TABLE II—CORRELATION COEFFICIENTS OF INGESTED NUTRIENTS WITH SERUM LIPID AND LIPOPROTEIN LEVELS IN CORONARY AND CONTROL SUBJECTS

Serum-fraction	Nutrient	Control (D.F. 82)	Coronary (D.F. 84)	Combined (control and coronary) (D.F. 168)
Total cholesterol	Total calories	−0·031	0·192	−0·006
	Total carbohydrate	−0·077	0·020	−0·123
	Sucrose	−0·084	−0·046	−0·107
	Carbohydrate (% sucrose)	−0·079	−0·107	−0·098
	Total fat	−0·069	0·226*	−0·024
	Fat (% calories)	−0·127	0·054	−0·087
	Eggs	0·137	0·145	0·115
	Milk fat	−0·100	0·154	−0·054
Ester cholesterol	Total fat	..	0·215*	..
Free cholesterol	Total carbohydrate	..	..	−0·157*
Phospholipid	Total calories	−0·248*	..	..
	Total carbohydrate	−0·402‡	..	−0·252†
	Sucrose	−0·373‡	..	−0·244†
	Carbohydrate (% sucrose)	−0·280†	..	−0·205†
	Total fat	..	0·236*	..
Standard serum-fraction:				
0–12 lipoprotein	Total calories	..	0·230*	..
12–20 lipoprotein	Total fat	..	0·229*	..
20–100 lipoprotein	Milk fat	..	..	−0·208†
100–400 lipoprotein	Total calories	−0·254*	..	..
	Total carbohydrate	−0·259*	..	..
	Total fat	−0·244*	..	..
	Fat (% calories)	..	..	−0·170*
	Milk fat	−0·297†	..	−0·221†
0–20 lipoprotein	Total calories	..	0·240*	..
	Total fat	..	0·232*	..
	Fat (% calories)	−0·234*	..	..
20–400 lipoprotein	Milk fat	−0·260*	..	−0·229†

The complete list of nutrients tested are shown under "total cholesterol". Although all were correlated with each lipid fraction, only the significant ones are shown in subsequent sections.
Probability level for r: $<0·05$ (*), $<0·01$ (†), $<0·001$ (‡).
D.F. = Degrees of freedom.

Table II shows the correlation coefficients of other nutrients with serum-lipids. None has such a consistent correlation as coffee.

Within the patient-group the correlations were calculated for each decade separately. The same trends were observed but the numbers of patients were too small to provide statistically significant results.

Discussion

These findings suggest that coffee in some way elevates serum-lipids in susceptible persons and that such persons may be prone to coronary heart-disease. A possible explanation is that coffee stimulates lipolysis with elevation of plasma unesterified fatty acid which is a substrate for hepatic synthesis of lipoproteins. The raised concentration of substrate may accelerate rate of synthesis and release of lipoproteins and raise serum-lipid levels. We were testing this hypothesis when Bellett et al. (1965) reported that two-and-a-half cups of "instant" coffee significantly raised plasma unesterified fatty acid levels in man during a three-hour period. Since intramuscular caffeine had the same effect they suggested that caffeine is the lipolytic ingredient of coffee.

In our earlier survey, the participants rarely used "instant" coffee. Other studies (unpublished) show that ground coffee also elevates plasma unesterified fatty acids. It has yet to be shown, however, that other serum-lipid fractions will become elevated with long-term drinking of coffee or that increased atherosclerosis results. Perhaps only certain people respond to elevated plasma unesterified fatty acids with an increase in lipoproteins. This might explain why coffee is correlated with serum-lipids only in people liable to coronary heart-disease. It is interesting to recall that adding coffee to the drinking-water caused a drastic and prolonged (two month) elevation of blood-sugar in obese mice but not in the normal animal (Kuftinec and Mayer 1964).

Our data suggest that substances in coffee other than caffeine may be important for hyperlipidæmia. Beverages made from tea leaves and ground coffee both contain 100–150 mg. of caffeine (Goodman and Gilman 1941, Turner 1959, Pearson 1962, Bender 1965), yet tea, unlike coffee, has no positive correlation with serum-lipids. Furthermore, the sum of the number of cups of coffee and tea has no significant correlations with serum-cholesterol in the coronary and control groups, separately or combined (Little et al. 1965). Extracts of coffee contain a number of substances (Turner 1959, Sivetz 1963), which might conceivably affect serum-lipid levels. Perhaps the effect of coffee may be partly in combination with certain other foods or with smoking.

The importance of the correlation coefficients in table I and II must be considered. A number of foods have significant correlations with one or two of the serum lipid or lipoprotein fractions. Although all are statistically significant, none is so significant as to make one confident of their biological importance. The correlation with coffee is consistently significant, however, and there is a positive correlation with every lipid and lipoprotein fraction in the patients examined.

Paul et al. (1963) found that Americans, who later developed coronary disease, drank more coffee than others. In our retrospective study of Canadians, however, coronary patients and controls drank equal amounts of coffee (Little et al. 1965). This difference in results cannot be explained unless, after the onset of coronary symptoms, patients drank less coffee. Toronto physicians have not, as a rule, prescribed modification of coffee ingestion in such cases. Unfortunately, we did not inquire specifically about any voluntary reduction of coffee consumption. Further investigation of the relation between coffee intake and the incidence of coronary heart-disease and serum-lipids is required.

Summary

Significant correlations between coffee drinking and serum lipid and lipoprotein concentrations were found in men with coronary heart-disease but not in healthy controls. Tea tended to have negative correlations with the serum lipids and lipoproteins in the patients. Other nutrients had no consistent correlation in either group. The data suggest that coffee contains a substance which

elevates serum-lipids in susceptible persons and that such persons may be liable to coronary heart-disease.

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Requests for reprints should be addressed to Dr. J. A. Little, Atherosclerosis Project, Sunnybrook Hospital, Toronto 12, Ontario, Canada.

REFERENCES

Bellett, S., Kershbaum, A., Aspe, J. (1965) *Archs intern. Med.* **116**, 750.

Bender, A. E. (1965) Dictionary of Nutrition and Food Technology; p. 35. London.

Bowes, A. de P., Church, C. F. (1951) Food Values of Portions Commonly Used. Philadelphia.

DeLalla, O. F., Gofman, J. W. (1954) Methods of Biochemical Analysis (edited by D. Glick); vol. 1, p. 459.

Goodman, L., Gilman, A. (1941) The Pharmacological Basis of Therapeutics; p. 283. New York.

Kuftinec, D. M., Mayer, J. (1964) *Metabolism*, **13**, 1369.

Little, J. A., Shanoff, H. M. (1963) *Can. med. Ass. J.* **89**, 961.

— Csima, A., Redmond, S. E., Yano, R. (1965) *Lancet*, **i**, 933.

Paul, O., Lepper, M. H., Phelan, W. H., Dupertuis, G. W., MacMillan, A., McKearn, H., Park, H. (1963) *Circulation*, **28**, 20.

Pearson, D. (1962) The Chemical Analysis of Foods. London.

Shanoff, H. M., Little, J. A. (1964) *Can. med. Ass. J.* **91**, 835.

— — Murphy, E. A., Rykert, H. E. (1961) *ibid.* **84**, 519.

Sivetz, M. S. (1963) Coffee Processing Technology; vol. 2. Westport, Connecticut.

Sperry, W. M., Webb, M. (1950) *J. biol. Chem.* **187**, 97.

Table of Food Values Recommended for Use in Canada (1951). Nutrition Division, Department of Health and Welfare, Ottawa, Canada.

Turner, D. F. (1959) Handbook of Diet Therapy; p. 38. Chicago.

Zilversmit, B. D., Davis, A. K. (1950) *J. Lab. clin. Med.* **35**, 155.

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CAFFEINE

by

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History

Chemists have exerted an extensive influence on Goethe and his portrait of nature. Besides Spielmann in Strassburg, Buchholz and Göttling in Weimar and Döbereine in Jena, Goethe was also closely associated with Friedrich Ferdinand Runge, a chemist and professor of technology in Breslau. As early as 1809, Runge had detected an alkaloid base in china-bark, and in 1834 he discovered aniline and phenol (carbolic acid) in coal-tar.

In 1820, in Goethe's presence, he undertook an experiment in which he demonstrated the effect of stramonium components on the eye of the cat. In gratitude and recognition, the city council presented him with a case of coffee beans, which the then still young researcher examined and became the first to discover the caffeine contained therein. The substance crystallized in the form of white needles, and Runge named it caffeine according to the Latin generic name of the coffee bush.

Caffeine was not synthesized until 75 years later by E. Fischer and Ach.

As it was not yet known that the active component of coffee and tea is the same, the alkaloid of coffee was named caffeine, and that of the tea-bush theine. Today, only the term caffeine is used.

Appearance

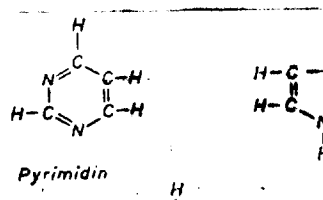
Caffeine is found up to 1-1.5% in coffee beans (Coffea arabica and C. liberica; Rubiaceae), up to 5% in black tea (Camellia sinensis, Theaceae), up to 1.5% in Brazil tea (Ilex paraguayensis, Caprifoliaceae), up to 1.5% in the cola nut, (Cola acuminata and C. vera, Sterculiaceae), and up to 6.5% in guarana paste (from the seeds of the South American Liane Paullinia cupana, Sapindaceae).

There are about 100-150 mg of caffeine in one cup of coffee.

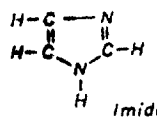
Chemistry

Caffeine is an alkaloid of the purine group. The so-called "purins" belong to the bicyclic heterosystems, which are compounds that are formed by condensation of two heterocycles.

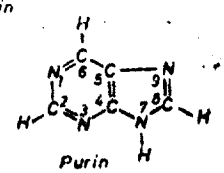
Purine itself is formed from one 6-member pyrimidin and one 5-member imidazol ring. Both compounds contain two nitrogen atoms in meta-position.



Pyrimidin

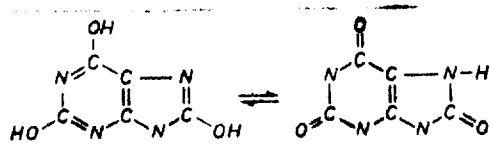


Imidazol



Purin

If we replace the three H atoms at the C atoms 2, 6 and 8 in purine with OH groups, then we obtain uric acid, a 2,6,8-dihydroxypurin, which can appear in two tautomeric forms:

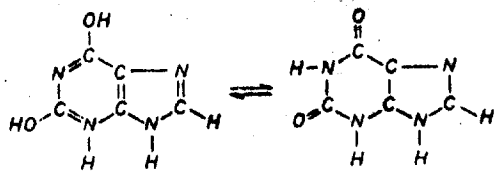


Uric acid (lactim form) Uric acid (lactam form)

By tautomery we mean the phenomenon when two isomeric compounds, which are in a certain balance, are distinguished by a change in position of a hydrogen atom, in the case of simultaneous shift of the bond.

Uric acid was discovered in bladder stones by Scheele in 1776. The human urine normally contains only small amounts of uric acid, though there can be formation of bladder and kidney stones that consist chiefly of the sodium or ammonia salt of uric acid. In the case of gout, uric acid is deposited in the joints.

Xanthine can be produced by heating uric acid. It is a 2,6-dihydroxypurin.

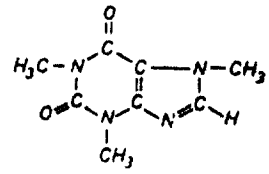


Xanthine (lactim form) Xanthine (lactam form)

As a physiological product of the protein metabolism, it appears in the liver, in the blood and in the urine, and is also found in smaller amounts in tea leaves, besides caffeine.

The three alkaloids theophyllin, theobromin and caffeine

are derived from the lactam form of xanthine by steplike replacement of the H atoms of the NH groups by methyl residues. Caffeine is a 1,3,7-trimethylxanthine:

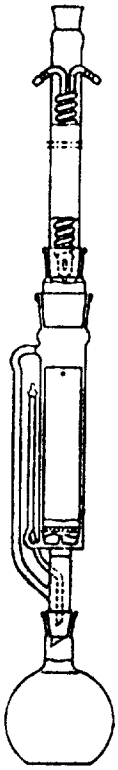


Caffeine

Preparation of Caffeine from Tea Leaves

The following working prescription is not of very great difficulty. It is supposed to give the practitioner the stimulus necessary to isolate this interesting natural substance himself and to prepare it purely. In a well-equipped chemical laboratory, this can be done easily.

In a soxhlet extraction apparatus (see figure) about 30 g of finely powdered black tea are extracted for eight hours with 200 ml 90% ethyl alcohol in a filter paper case, which can be made easily. For a good yield, it is important that the tea is ground as finely as possible beforehand. After the extraction time, one prepares in a porcelain bowl a suspension of 50 g magnesium oxide in 300 ml water, adds the alcohol extract and steams over a water-bath, agitating frequently, until it is dry. Then the powdered residue is boiled first with 250 ml, then with 125 ml water, and then sunctioned off hot through a porcelain frit. The combined filtrates are evaporated to about one third after addition of 25 ml diluted sulfuric acid (16%). Here, a flaky precipitate is sometimes separated, which precipitate should be filtered off. After cooling the liquid,



it is shaken out five times in a separating funnel with 30 ml of chloroform each time. For purification of the yellow-colored chloroform solution, it is shaken out with several ml caustic soda solution (15%), washed with about the same amount of water, separated, and the chloroform phase evaporated. The residue, consiting of raw caffeine, can be recrystallized with alcohol or with a little hot water, and thus purified. One obtains a white, very fine, crystalline, oderless, bitter-tasting powder.

Detection of Caffeine in Black Tea

a) By means of microsublimation

If one heats some ground tea leaves carefully with the pilot light of a Bunsen Burner between two slides or two watch-glasses on an asbestos wire net, then after a short time, on the underside of the upper glass, the long needles of caffeine appear.

b) By means of murexide reaction

Here, first of all, one produces a chloroform extract from tea leaves by extracting a small amount of the drug with chloroform using a magnetic agitator or by shaking. The filtered off chloroform solution is evaporated to dryiness, a knife-tip amount of the residue is put in a porcelain bowl, a few drops of diluted hydrogen peroxide solution and a few drops of diluted hydrochloric acid are added (see also medical text monographs on caffeine) and this is evaporated to dryness over the water-bath. There remains a yellow or yellowish-red blotch, which is colored to purplish-red when dampened with ammonia solution as murexide is formed (murex = purpura).

We will not enter here into the structure formula of murexide.

However, it should be mentioned that all purins (e.g. uric acid, xanthine and the alkaloids theophyllin, theobromin and caffeine) manifest this detection reaction. Nonetheless, chemically different "murexides" form. From uric acid and xanthine there forms a methyl-free murexide, from the alkaloids, a methylized murexide.

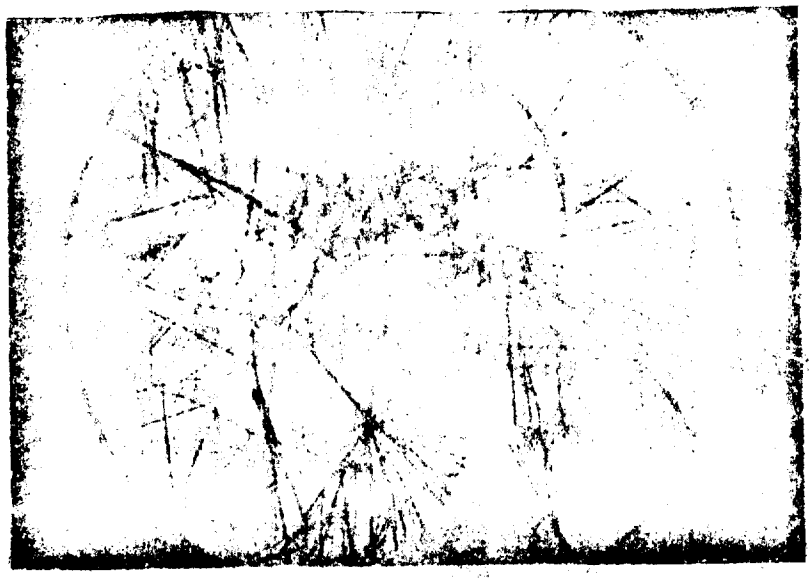


Fig. 2: Caffeine crystals. Enlarged 320 x.

Pharmacology

Caffeine has two chief effects: first the diuretic, urine-coaxing effect, and second, the central-stimulatory effect. It is interesting to compare the effects of caffeine with those of the two other purin alkaloids: all three alkaloids have a diuretic effect, that of theophyllin being strongest. Generally, only this last is applied as a diuretic, because its central stimulatory effect is slight, in contrast to caffeine. With respect to the diuretic effect, theobromin stands between theophyllin and caffeine; the central-stimulatory effect is nearly completely lacking in theobromine. In the case of caffeine, diuresis is clearly intensified by alcohol consumption.

		A. Diurese	B. zentrale Erregung
1.	Theophyllin	+ + +	+
2.	Theobromin	+ +	
3.	Coffein	+	+ + +

Key:

A. diuresis B. central stimulation
1. theophylline 2. theobromine 3. caffeine

Caffeine is a very effective, as well as relatively harmless excitant. Its effect is based on a stimulation of the central nervous system. In this stimulation, it moves from the cerebral cortex gradually to the nerve centers in the spine marrow. The respiratory center is stimulated, whereby the respiration is accelerated and deepened. The blood vessels of the brain and the skin, as well as the coronary vessels nourishing the heart are dilated, while the blood vessels of the abdominal organs are constricted. Beyond this, there is an indirect effect on the heart, which is stimulated, and its achievement increased. This increase in operation is based on an intensification of the contraction phase of the heart (systole). The rest pauses of the heart (diastoles) are not, however, lengthened, as with digitalis, but are, on the contrary, shortened. The "heart pump" thus operates under the influence of caffeine with greater pressure and shorter "piston" action. Blood pressure and blood circulation velocity increase, and the basal metabolism rises.

The usual therapeutic oral dose is 100-250 mg. Too large caffeine doses (330 mg and upwards) cause the hands to shake and produce a rush of blood to the head and pressure in the area of the heart. The lethal dose appears to be quite high; after intake

of 5 g caffeine, cases of survival have been described.

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Aus dem Arbeitstagebuch eines Praktikanten

Das Coffein

Von Klaus T. Löffler, Praktikant der Marien-Apotheke Stuttgart

Geschichte

Apotheker haben auf Goethe und dessen Bild von der Natur nachhaltigsten Einfluß ausgeübt. Neben Spielmann in Straßburg, Buchholz und Göttling in Weimar sowie Döbereine in Jena, stand Goethe auch in enger Verbindung mit Friedrich Ferdinand Runge, Apotheker und Professor der Technologie in Breslau. Dieser hatte bereits 1809 in der Königschinarinde eine Alkaloidbase nachgewiesen und entdeckte 1834 Anilin und Phenol (Carbolsäure) im Steinkohlenteer.

Im Jahre 1820 unternahm er in Anwesenheit von Goethe ein Experiment, bei dem er die Wirkung der Stechapfelinhaltsstoffe auf das Auge einer Katze demonstrierte. Als Dank und Anerkennung schenkte ihm der Geheimrat ein Kästchen mit Kaffeebohnen, die der damals noch junge Forscher untersuchte und dabei als erster das darin enthaltene Coffein entdeckte. Der Stoff kristallisierte in weißen Nadeln aus und Runge benannte ihn nach der lateinischen Gattungsbezeichnung des Kaffeestrauches Coffein.

Erst 75 Jahre später erfolgte durch E. Fischer und Ach die Synthese des Coffeins.

Als man noch nicht wußte, daß der wirksame Inhaltsstoff des Kaffees und des Tees identisch ist, nannte man das Alkaloid des Kaffees Coffein, das des Teestrauchs Thein. Heute wird nur noch die Bezeichnung Coffein benutzt.

Vorkommen

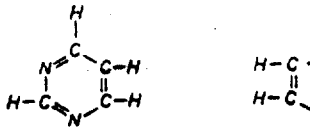
Coffein findet sich in den Kaffeebohnen (Coffea arabica und C. liberica, Rubiaceae) zu 1—1,5 %, im schwarzen Tee (Camellia sinensis, Theaceae) bis zu 5 %, im Mate-Tee (Ilex paraguayensis, Caprifoliaceae) bis zu 1,5 %, in der Kolanuß (Cola acuminata und C. vera, Sterculiaceae) bis zu 1,5 % und in der Guaranapaste (aus den Samen der südamerikanischen Liane Paullinia cupana, Sapindaceae) bis zu 6,5 %.

In einer Tasse Kaffee sind ca. 100—150 mg Coffein enthalten.

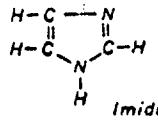
Chemie

Coffein ist ein Alkaloid der Puringruppe. Die sog. „Purine" gehören zu den bicyclischen Heterosystemen, das sind Verbindungen, die durch Kondensation zweier Heterocyclen entstanden sind.

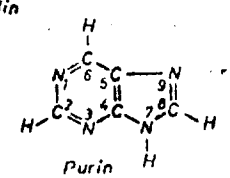
Purin selbst baut sich aus einem 6gliedrigen Pyrimidin- und einem 5gliedrigen Imidazolring auf. Beide Verbindungen enthalten zwei Stickstoffatome in Meta-Stellung.



Pyrimidin

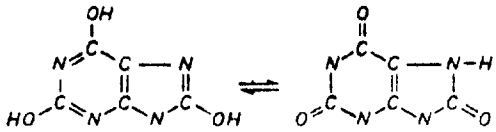


Imidazol



Purin

Denkt man sich im Purin die drei H-Atome an den C-Atomen 2, 6 und 8 durch OH-Gruppen ersetzt, so gelangt man zur **Harnsäure**, einem 2,6,8-Dihydroxypurin, die in zwei tautomeren Formen auftreten kann:

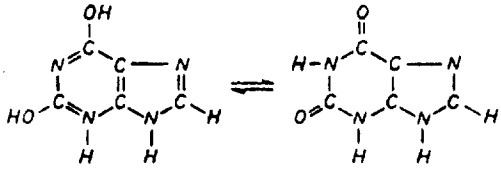


Harnsäure (Lactimform) Harnsäure (Lactamform)

Unter Tautomerie versteht man die Erscheinung, daß sich zwei isomere Verbindungen, die in einem bestimmten Gleichgewicht stehen, durch Platzwechsel eines Wasserstoffatoms bei gleichzeitiger Verschiebung der Bindung unterscheiden.

Harnsäure wurde 1776 von Scheele in Blasensteinen entdeckt. Der menschliche Harn enthält normalerweise Harnsäure nur in geringen Mengen, jedoch kann es zur Bildung von Blasen- und Nierensteinen kommen, die überwiegend aus dem Natrium- oder Ammoniumsalz der Harnsäure bestehen. Bei der Gicht wird Harnsäure in den Gelenken abgelagert.

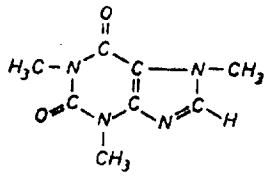
Durch Erhitzen von Harnsäure kann **Xanthin** hergestellt werden. Es ist ein 2,6-Dihydroxypurin.



Xanthin (Lactimform) Xanthin (Lactamform)

Als physiologisches Produkt des Eiweißstoffwechsels kommt es in der Leber, im Blut und im Harn vor, in geringer Menge findet es sich auch neben dem Coffein in den Teeblättern.

Von der Lactamform des Xanthins leiten sich die drei Alkaloide **Theophyllin**, **Theobromin** und **Coffein** durch stufenweisen Ersatz der H-Atome der NH-Gruppen durch Methylreste ab. Das Coffein ist ein 1,3,7-Trimethylxanthin:



Coffein

Darstellung des Coffeins aus Teeblättern

Die nachfolgend gegebene Arbeitsvorschrift besitzt keinen großen Schwierigkeitsgrad. Sie soll dem Praktikanten einen Anreiz geben, diesen interessanten Naturstoff selbst zu isolieren und rein darzustellen. In einem gut eingerichteten Apothekenlabor ist dies ohne weiteres möglich.

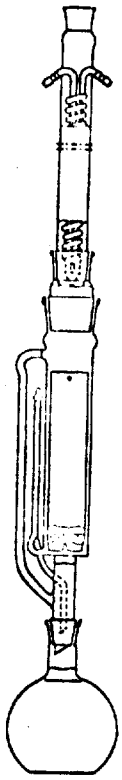


Abb. 1:

In einem Soxhlet-Extraktionsapparat (siehe Abbildung 1) werden ca. 30 g feingepulverter schwarzer Tee acht Stunden lang mit 200 ml Aethylalkohol 90 % in einer Filtrierpapierhülse, die man sich ohne weiteres selbst herstellen kann, extrahiert. Entscheidend für eine gute Ausbeute ist, daß man den Tee vorher so fein wie möglich zerkleinert. Nach der Extraktionszeit bereitet man sich in einer Porzellanschale eine Aufschwemmung von 50 g Magnesiumoxid in 300 ml Wasser, gibt den alkoholischen Extrakt dazu und dampft auf dem Wasserbad unter häufigem Umrühren zur Trockne ein. Dann wird der pulverige Rückstand zunächst mit 250 ml, anschließend mit 125 ml Wasser ausgekocht und heiß durch eine Porzellantritte abgesaugt. Die vereinigten Filtrate werden nach Zusatz von 25 ml verdünnter Schwefelsäure (16 %) auf etwa ein Drittel eingedampft. Hierbei scheidet sich manchmal ein flockiger Niederschlag aus, den man abfiltriert. Nach Abkühlung der Flüssigkeit schüttelt man diese fünfmal in einem Scheidetrichter mit je 30 ml Chloroform aus. Zur Reinigung der gelb gefärbten Chloroformlösung wird diese mit einigen ml Natronlauge (15 %) geschüttelt, mit etwa ebensoviel Wasser gewaschen, abgetrennt und die Chloroformphase eingedampft. Der Rückstand, bestehend aus Rohcoffein, kann nur mit Alkohol oder mit wenig heißem Wasser umkristallisiert und dadurch gereinigt werden. Man erhält ein weißes, sehr feines, kristallines, geruchloses, bitter schmeckendes Pulver.

Nachweis des Coffeins im schwarzen Tee

a) Durch Mikrosublimation

Erhitzt man einige pulverisierte Teeblätter vorsichtig mit der Sparflamme des Bunsenbrenners zwischen zwei Objektträgern oder zwei Uhrgläschen auf einem Asbestdrahtnetz, dann erscheinen nach kurzer Zeit auf der Unterseite des oberen Glases die langen Nadeln des Coffeins.

b) Durch die Murexidreaktion

Hierzu stellt man sich zuerst einen Cloroformauszug aus Teeblättern in der Weise her, daß man eine kleinere Menge der Droge mit Chloroform zweckmäßigerweise mittels Magnetrührer oder durch Schütteln extrahiert. Die abfiltrierte Chloroformlösung dampft man zur Trockne ein, bringt eine Messerspitze des Rückstandes in ein flaches Porzellanschälchen, gibt einige Tropfen verdünnte Wasserstoffperoxidlösung und einige Tropfen verdünnte Salzsäure hinzu (siehe auch Arzneibuchmonographie über Coffein) und dampft auf dem Wasserbad erneut zur Trockne ein. Es bleibt ein gelber bis gelbroter Fleck zurück, der sich beim Befeuchten mit Ammoniaklösung durch Bildung von Murexid purpurrot färbt (murex = Purpurschnecke).

Auf die Strukturformel des Murexids soll hier nicht eingegangen werden. Es soll jedoch erwähnt werden, daß alle Purine (z. B. Harnsäure, Xanthin und die Alkaloide Theophyllin, Theobromin und Coffein) diese Nachweisreaktion geben. Allerdings enstehen chemisch unterschiedliche „Murexide". Aus Harnsäure und Xanthin ensteht ein methylfreies, aus den Alkaloiden entstehen methylierte Murexide.

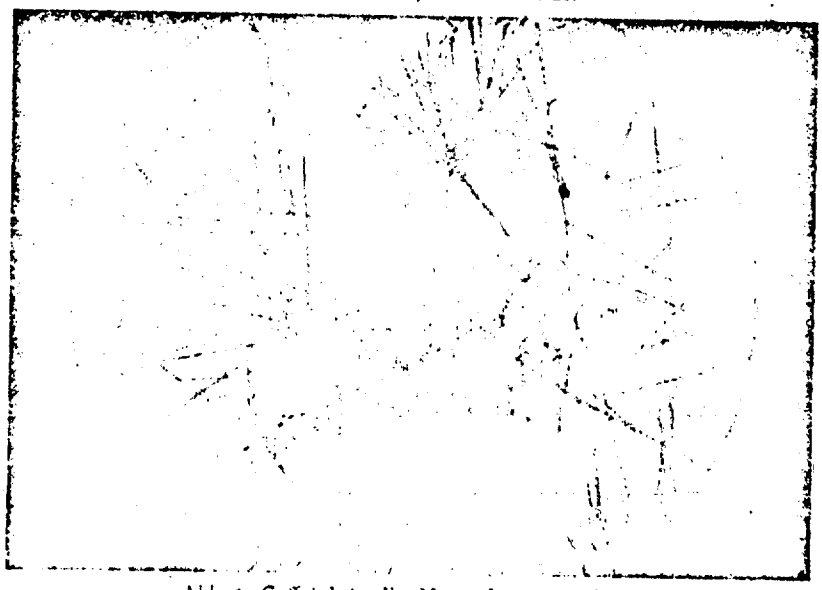


Abb. 2: Coffeinkristalle. Vergrößerung 320fach.

Pharmakologie

Das Coffein besitzt zwei hauptsächliche Wirkungen: Einmal die diuretische, harntreibende Wirkung, zum anderen den zentralerregenden Effekt. Interessant ist es, die Wirkungen des Coffeins mit denen der beiden anderen Purinalkaloide zu vergleichen: Alle drei Alkaloide wirken diuretisch, am stärksten das Theophyllin. Als Diuretikum wird im allgemeinen nur dieses eingesetzt, weil sein zentral erregender Effekt im Unterschied zum Coffein gering ist. Hinsichtlich der diuretischen Wirkung steht das Theobromin zwischen dem Theophyllin und dem Coffein, die zentralerregende Wirkung fehlt dem Theobromin nahezu ganz. Beim Coffein wird die Diurese durch Alkoholgenuß deutlich verstärkt.

	Diurese	zentrale Erregung
Theophyllin	+ + +	+
Theobromin	+ +	
Coffein	+	+ + +

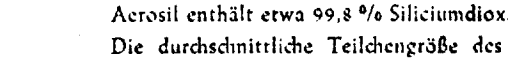
Coffein ist ein gut wirksames, dabei relativ harmloses Excitans. Seine Wirkung beruht auf einer Anregung des Zentralnervensystems. Dabei greift es von der Großhirnrinde ausgehend allmählich auf die Nervenzentren des Rückenmarks über. Das Atemzentrum wird erregt, dadurch kommt es zu einer Beschleunigung und Vertiefung der Atmung. Die Blutgefäße des Gehirns und der Haut sowie die das Herz ernährenden Kranzgefäße werden erweitert, die Blutgefäße der Bauchorgane jedoch verengt. Darüber hinaus kommt es zu einem unmittelbaren Einfluß auf das Herz, das angeregt und in seiner Leistung gesteigert wird. Diese Leistungssteigerung beruht auf einer Verstärkung der Kontraktionsphase des Herzens (Systole). Die Ruhepause des Herzens (Diastole) wird allerdings nicht wie bei Digitalis verlängert, sondern im Gegenteil verkürzt. Die „Herzpumpe" arbeitet also unter dem Einfluß von Coffein mit größerem Druck und kürzerem „Kolben"weg. Blutdruck und Blutumlaufgeschwindigkeit nehmen zu, der Grundumsatz steigt.

Die übliche therapeutische orale Gabe beträgt 100—250 mg. Zu hohe Coffeindosen (von 300 mg aufwärts) rufen Händezittern, Blutandrang nach dem Kopf und Druck in der Herzgegend hervor. Die Dosis letalis scheint recht hoch zu liegen: Nach Einnahme von 5 g Coffein wurden Überlebensfälle beschrieben.

AEROSIL® — ein vielseitiger Hilfsstoff der Galenik

Von Karin Runge, Braunschweig

Aerosil ist das eingetragene Warenzeichen der Firma Degussa in Frankfurt am Main. Es handelt sich um eine amorphe, hochdisperse, kolloide Kieselsäure von höchster Reinheit. Sie wird dargestellt durch Flammenhydrolyse von gasförmigem Siliciumtetrachlorid:



$$SiCl_4 + 2\,H_2O \rightarrow SiO_2 + 4\,HCl$$

Aerosil enthält etwa 99,8 % Siliciumdioxid.

Die durchschnittliche Teilchengröße des Aerosils beträgt 10—30 µm (Mikrometer). Dispergiert man Aerosil in Wasser, so entsteht ein hydrophiles Kolloid. Hierbei wirkt die Umhüllung mit Wassermolekülen („Hydratation") stabilisierend auf die kolloidal gelösten Teilchen, denn hydrophile Kolloide haben ein großes Bestreben, Wassermoleküle zu absorbieren. Diese wiederum verhindern die Vereinigung der Kolloidteilchen zu gröberen Partikeln. Das Bestreben zur Anlage von Wasser geht so weit, daß das entstandene Sol zu einer gallertartigen Masse, dem Gel, erstarrt. Durch Rühren oder Schütteln kann dieses Gel wieder zu einem Sol geformt werden (reversibles Kolloid). Diese Thixotropie hängt von der Art des flüssigen Systems ab. Je unpolarer dieses ist, desto größer ist die Thixotropie. Entscheidend ist auch die Art der Einarbeitung: Glasstab, Pistill- oder elektrisches Rührwerk.

Um zu sehen, wie sich das Aerosil in den verschiedenprozentigen Wassergemischen verhält und wieviel Prozent des Standard-Aerosils nötig sind, um eine Salbenkonsistenz zu erreichen, stellten wir eine Versuchsreihe von 17 Versuchen der verschiedenprozentigen Aerosil-Wassergemische auf. Die Arbeiten wurden in einer Kunststoff-Fantaschale mit Kunststoffpistill durchgeführt. Die Ergebnisse sind in folgender Tabelle zusammengefaßt:

Marek-'67
Bib. No. 2065

Acta Univ. Palacki Olomuc Fac. Med. 44:101-112.
1967.

1967 - ACTA UNIVERSITATIS PALACKIANAE OLOMUCENSIS - TOM. 44
FACULTATIS MEDICAE

Medical Faculty of the Palacký University, Olomouc, Czechoslovakia
Department of Pharmacology
Director: Prof. MUDr. Jiří Lenfeld, CSc.

A CONTRIBUTION TO THE EFFECT OF CAFFEINE ON SOME EXPERIMENTALLY INDUCED OEDEMAS IN RATS

J. MAREK

We have found already previously that a dose of 100 mg/kg of caffeine applied subcutaneously suppresses formaldehyde-induced oedemas in rats (Lenfeld 1958) and oedemas induced by egg white, hyaluronidase and dextran (Marek, Lenfeld, Jezdinský 1961). In our further experiments the mechanism of its action has been followed up (Lenfeld, Stojan 1961, Marek, Lenfeld, Jezdinský 1961, Lenfeld, Kroutil 1961, Lenfeld, Sládková, Grundman 1963, Lenfeld, Dušek, Jezdinský 1965).

The present study has been carried out to extend our knowledge concerning the effect of caffeine on experimental oedemas, its purpose being especially to explain the problem whether the anti-inflammatory effect of caffeine on experimental oedemas following subcutaneous application is not due to non-specific stressing irritation in site of injection and whether it is reproducible also following a single dose given orally.

MATERIAL AND METHODS

Groups of white rats, delivered by Velaz n. p., of average body weight ranging between 100-240 g, both male and female, kept on a standard laboratory diet, were employed in our experiments.

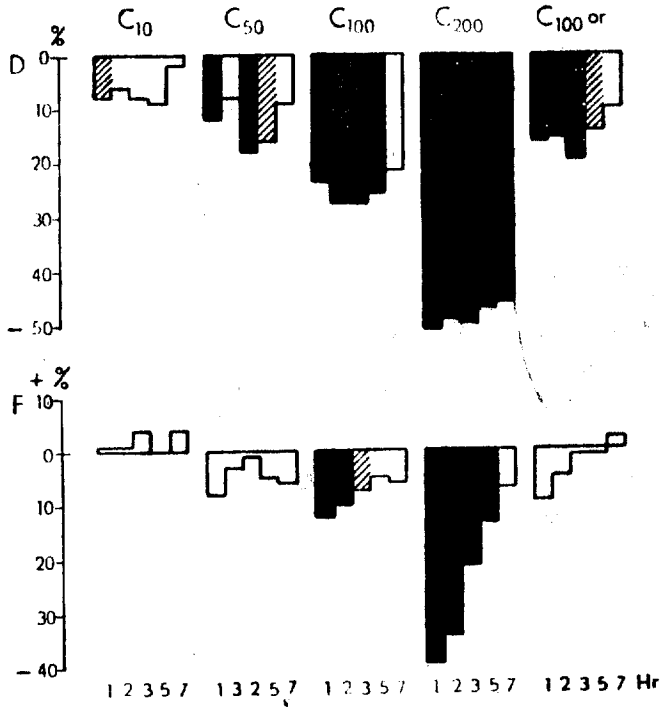
Oedemas were induced by 0.1 ml of 6 % dextran (Dextran Spofa), 0.2 ml of 2 % aqueous solution of formaldehyde, 0.2 ml of fresh egg white (diluted in saline 1 : 10), 0.05 ml of 1 % suspension of carrageenin prepared in saline (Sea Kem 402*), and 0.1 ml of 10 % aqueous suspension of kaolin. These irritants were prepared always immediately before the experiment and injected under the palmar surface of the hind paw. The oedemas were measured in the dorsoplantar dimension by a slide gauge in 1/10 mm in the first, second, third, fifth, and seventh hours of the oedema. From this size the normal size of the paw was

* Obtained through the courtesy of Marine Colloids, Inc., Springfield, N. J., USA.

substracted, the result being taken for the size of an oedema caused by a phlogistic agent.

Besides, a method using carrageenin-induced abscess as a model of acute exudative inflammation (according to Benitz and Hall 1963) was used: 0.5 ml of 2 % aqueous suspension of carrageenin was injected subcutaneously into the back of the animal, the abscess being dissected and weighed after 24 hours. Finally, a method of testing for anti-inflammatory activity was employed which is based on weighing granular tissue formed around cotton pellets implanted under aseptic conditions under the dorsal skin of the animal (according to Meier, Schuler and Dessaulles 1950).

In some cases individual models of inflammation were combined in one and the same animal, as they do not significantly influence each other, which had been shown by Kroutil (1964), so that dextran was injected into one paw and egg white into the contralateral one, or dextran into one paw and formaldehyde into the contralateral one. Carrageenin-induced abscess was sometimes tested in



Graph 1. Percentual expression of the suppression of dextran (D) and formaldehyde (F) oedema of the rat hind paw due to subcutaneous administration of caffeine at various dose levels - C_{10} = 10 mg/kg of caffeine, C_{50} = 50 mg/kg of caffeine, C_{100} = 100 mg/kg of caffeine, C_{200} = 200 mg/kg of caffeine. For comparison 100 mg/kg of caffeine was administered to one group of rats orally (C_{100}or). Black columns = $p < 0.02$, shaded columns = $p < 0.05$, white columns = $p > 0.05$, – % = improvement, + % = deterioration as compared with the controls.

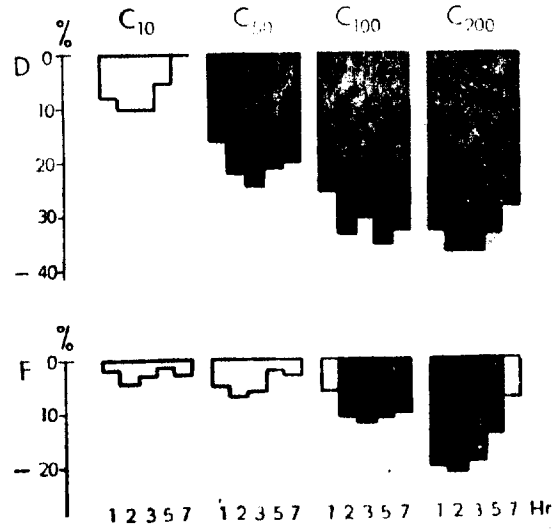
rats, which obtained simultaneously dextran into the left hind paw and formaldehyde into the right hind paw.

The number of animals equaled in individual groups in most cases 10. Antiphlogistic agents under study were given, unless otherwise indicated, always in a single dose by a stomach tube 60 min. before the experiment, or in a single subcutaneous injection 30 min. before the experiment. All drugs were applied in a volume of 1 ml per 100 g body weight. An aqueous solution of caffeine (coffeinum purum) was applied subcutaneously and orally at various dose levels (10, 50, 100, 200 mg per kg body weight). The controls were treated with the same volume of saline. The results were estimated by means of Student's t-test and expressed in graphs: black columns mean highly significant values ($p < 0.02$), shaded columns represent values approaching the level of significance ($p < 0.05$), white columns represent insignificant values ($p > 0.05$). The tables show average values of oedemas measured in 1/10 mm $\pm$ e (standard error) and the number of animals. The experiments were performed during the period from July 1960 to January 1966.

EXPERIMENTS

I. Effect of various subcutaneous doses of caffeine on experimental oedemas induced by dextran and formaldehyde and on carrageenin-induced abscess

Experimental oedemas were induced in the left hind paws of male rats by dextran, in the right hind paws by formaldehyde, and simultaneously carrageenin



Graph 2. Percentual expression of the suppression of dextran (D) and formaldehyde (F) oedema of the rat hind paw due to oral administration of caffeine at various dose levels - C_{10} = 10 mg/kg of caffeine, C_{50} = 50 mg/kg of caffeine, C_{100} = 100 mg/kg of caffeine, C_{200} = 200 mg/kg of caffeine. Black columns = $p < 0.02$, shaded columns = $p < 0.05$, white columns = $p > 0.05$

was injected subcutaneously into the rostral region of the animals. Forty five minutes before the experiment the rats were pretreated with caffeine in various doses 10, 50, 100 and 200 mg/kg subcutaneously and with 100 mg/kg of caffeine orally. All animals were regularly mixed in animal cages. The oedemas were measured in the above mentioned way, the animals were sacrificied after 24 hours by ether inhalation, the carrageenin-induced abscesses were dissected and weighed.

The results are summarized in Table 1, Graph 1 and 3.

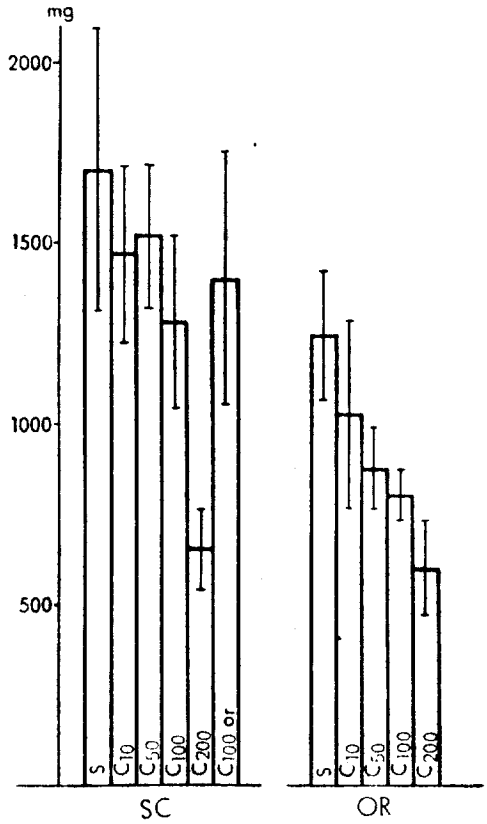
It is obvious from the results obtained that with an increasing dose of caffeine its inhibitory effect on the size of oedemas and on carrageenin-induced abscess increases as well. The weight of carrageenin-induced abscess following a dose of

	Hours	1		2		3		5		7		*
	Treatment	$\bar{x}$	± e	$\bar{x}$	± e	$\bar{x}$	± e	$\bar{x}$	± e	$\bar{x}$	± e	
DEXTRAN	Controls sc.	46.0	±1.4	43.0	±1.6	37.3	±1.2	31.3	±1.5	22.9	±2.1	10
	Caffeine 10 mg/kg sc.	42.1	±1.2	40.6	±1.0	34.4	±1.0	28.6	±0.4	22.4	±0.7	10
	Caffeine 50 mg/kg sc.	40.5	±1.4	39.5	±1.2	30.7	±1.4	26.3	±1.4	20.9	±1.0	10
	Caffeine 100 mg/kg sc.	35.5	±2.2	31.3	±2.2	27.3	±1.5	23.1	±1.5	18.1	±1.5	10
	Caffeine 200 mg/kg sc.	22.8	±1.5	22.2	±1.7	19.1	±0.9	16.8	±1.5	12.7	±1.1	10
	Caffeine 100 mg/kg or.	38.7	±1.4	36.5	±1.1	30.3	±1.1	27.0	±0.8	20.6	±0.9	10
FORMALDEHYDE	Controls sc.	39.3	±1.2	46.7	±1.3	49.9	±1.2	53.3	±1.8	51.9	±1.7	10
	Caffeine 10 mg/kg sc.	39,9	±1.8	47.4	±1.5	51.9	±1.5	53.5	±1.2	54.1	±1.5	10
	Caffeine 50 mg/kg sc.	36.3	±1.8	45.1	±0,9	49.5	±1.2	50.6	±1.5	48.9	±1.9	10
	Caffeine 100 mg/kg sc.	34.6	±0.9	42.1	±1.1	46.4	±1.1	50.5	±1.3	48,9	±1.8	10
	Caffeine 200 mg/kg sc.	24.0	±1.3	30.9	±1.4	39.4	±1.9	46.5	±1.2	48.3	±1.2	10
	Caffiene 100 mg/kg or.	35.9	±1.3	44.2	±1.5	49.6	±1.5	52.7	±1.4	52.9	±1.5	10

* = No. of animals

Table 1 Effect of subcutaneous administration of caffeine at various dose levels on dextran and formaldehyde oedemas - $\bar{x}$ = average size of oedema in 1/10 mm, ± e = standard error

10 mg/kg of caffeine decreased by 14 % ($p < 0.3$), following 50 mg/kg of caffeine by 10 % ($p < 0.4$), following 100 mg/kg of caffeine by 24 % ($p < 0.1$), and following 200 mg/kg of caffeine even by 61 % ($p < 0.01$). Oral dosing with 100 mg/kg of caffeine revealed also anti-inflammatory activity especially on dextran-induced oedema, though not so strong as compared with that after analogous dose given subcutaneously. Peroral treatment of carrageenin-induced abscess with 100 mg/kg of caffeine caused its suppression by 18 %, this being, however, statistically insignificant ($p < 0.3$). The rats treated with the highest dose of caffeine had the paws with formaldehyde-induced oedemas apparently more redish than the controls and than those treated with lower doses of caffeine. One animal from this group died after 24 hours and was not included into the evaluation of carrageenin-induced abscess.



Graph 3 Effect of caffeine given subcutaneously (SC) and orally (OR) at various dose levels on the weight of carrageenin induced abscess in mg. - S = control group treated with saline, C_{10} = 10 mg/kg of caffeine, C_{50} = 50 mg/kg of caffeine, C_{100} = 100 mg/kg of caffeine, C_{200} = 200 mg/kg of caffeine, C_{100} or = control group administered 100 mg/kg of caffeine orally. Confidence intervals for P = 95 are given in the graph. Number of animals: SC: C_{10} = 9, C_{50} = 10, C_{100} = 10, C_{200} = 9, S = 8, C_{100} or = 9; OR: C_{10} = 8, C_{50} = 10, C_{100} = 10, C_{200} = 10, S = 8.

II. Effect of various oral doses of caffeine on experimental oedemas induced by dextran and formaldehyde and on the weight of carrageenin-induced abscess.

Female rats were used in an analogous experiment, caffeine was administered at various dose levels by a stomach tube 60 min. before the experiment.

The results are shown in Table 2 and in Graphs 2 and 3.

The results obtained show caffeine, even when given orally, to suppress these models of inflammation again in dependence on the dose. The effect of caffeine on dextran-induced oedema was found statistically significant only for 50 mg/kg and for higher doses, a dose of 200 mg/kg did not have more pronounced effect than the preceding one. Formaldehyde-induced oedema, which is caused by a relatively strong irritant, could not have been influenced by lower doses of caffeine, the difference between the effects of 100 and 200 mg/kg shows still the dependence on the dose. The weight of carrageenin-induced abscess, when the rats are treated with increasing doses of caffeine, reveals apparently a decreasing

	Hours	1		2		3		5		7		*
	Treatment	$\bar{x}$	±e	$\bar{x}$	±e	$\bar{x}$	±e	$\bar{x}$	±e	$\bar{x}$	±e	
DEXTRAN	Controls or.	42.0	±1.1	37.5	±1.1	33.2	±0.8	29.0	±1.0	24.7	±0.7	10
	Caffeine 10 mg/kg or.	38.7	±1.6	33.9	±1.7	29.9	±1.5	27.5	±1.5	24.6	±1.6	10
	Caffeine 50 mg/kg or.	35.3	±1.7	29.1	±1.7	25.1	±1.1	23.0	±1.2	19.8	±1.1	10
	Caffeine 100 mg/kg or.	31.3	±1.7	25.1	±1.1	23.1	±1.8	18.8	±1.1	16.9	±1.4	10
	Caffeine 200 mg/kg or.	28.5	±1.8	23.9	±1.5	21.3	±1.3	19.5	±1.2	17.8	±1.1	10
FORMALDEHYDE	Controls or.	36.2	±1.4	44.5	±1.0	50.4	±1.1	52.5	±1.0	53.1	±1.2	10
	Caffeine 10 mg/kg or.	35.4	±2.3	42.6	±2.1	49.1	±1.7	51.8	±1.7	51.6	±1.7	10
	Caffeine 50 mg/kg or.	34.4	±2.2	41.3	±1.9	47.4	±1.3	51.6	±0.9	51.5	±1.7	9
	Caffeine 100 mg/kg or.	34.0	±1.4	39.6	±0.9	44.5	±0.9	46.9	±1.0	47.8	±0.9	10
	Caffeine 200 mg/kg or.	28.9	±1.5	35.3	±2.2	40.9	±2.5	45.1	±1.9	49.2	±1.5	10

* = No. of animals

Table 2. Effect of oral administration of caffeine at various dose levels on dextran and formaldehyde oedemas - $\bar{x}$ = average size of oedema in 1/10 mm, ±e = standard error

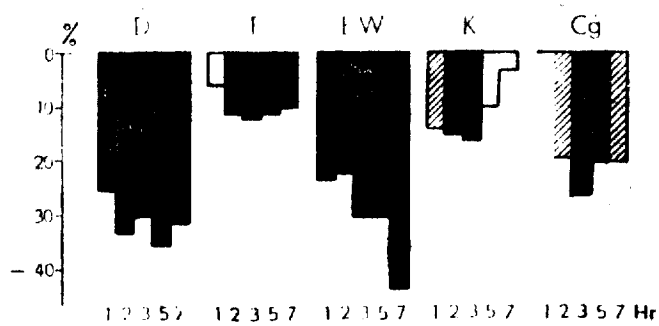
tendency: 10 mg/kg of caffeine suppresses this weight by 17 %, ($p < 0.2$), 50 mg/kg by 29 % ($p < 0.01$), 100 mg/kg by 35 % ($p < 0.01$), 200 mg/kg by 51 % ($p < 0.01$), the difference in the effects of the first three doses being not significant. Also in this experiment toxic effect of the highest dose of caffeine resulted in a death of one animal after 24 hours. Vasodilatation effect of this highest dose of caffeine could be also seen in the colour of the paw with formaldehyde-induced oedema.

In further experiments a dose of 100 mg/kg of caffeine was administered either subcutaneously or orally. These experiments were carried out to test for anti-inflammatory effect of this dose of caffeine on some other models of inflammation and to determine its effect following long term pretreatment of rats with caffeine with respect to the possibility of habit and tolerance of the organism towards caffeine.

III. Effect of a dose of 100 mg/kg of caffeine on experimental oedemas provoked by various phlogistic agents in the rat hind paw

The oedemas were induced by egg white, formaldehyde, dextran, kaolin, and carrageenin. Typical results were chosen from various experiments and expressed in the percentage of improvement of oedema.

The results are summarized in Graph 4.



Graph 4. Percentual comparison of antiexudative effect of 100 mg/kg of caffeine given orally on experimental oedemas induced by dextran (D), formaldehyde (F), egg white (EW), kaolin (K) and carrageenin (Cg). - Black columns = $p < 0.02$, shaded columns = $p < 0.05$, white columns = $p > 0.05$. The values of carrageenin-induced oedema were not recorded in the first hour.

IV. Effect of repeated oral administration of caffeine on experimental oedemas induced by dextran and formaldehyde as compared with the effect of a single dose of caffeine

Oedemas were induced in the left hind paws of the rats by dextran, in the right hind paws by formaldehyde. The first group was given daily for 7 days before the experiment and then 45 min. before inducing the oedema 100 mg/kg of caffeine

orally (8C), the second group was treated in the same way with saline (8S). The third group was administered only a single oral dose of caffeine (1C) 45 min. before the experiment, the fourth group was treated with a single dose of saline (1S).

The results are given in Table 3 and Graph 5a.

There was nearly no difference in the development of oedemas between both the control groups (1S and 8S), only in the third hour the development of formaldehyde-induced oedema was significantly milder in those rats treated with saline repeatedly. On the contrary, however, there was a statistically significant difference between the development of oedemas treated with a single dose of caffeine and those treated with caffeine repeatedly: repeated administration of caffeine suppressed dextran-induced oedema to a lesser degree than a single dose did, while the development of formaldehyde-induced oedema after repeated administration was even deteriorated. The suppression of dextran-induced oedema after repeated administration was from the third hour significantly less pronounced by 14-22 % than after a single dose, the development of formaldehyde-induced oedema was after repeated administration from the second hour significantly deteriorated (by 14-6 %) as compared with the effect of a single dose.

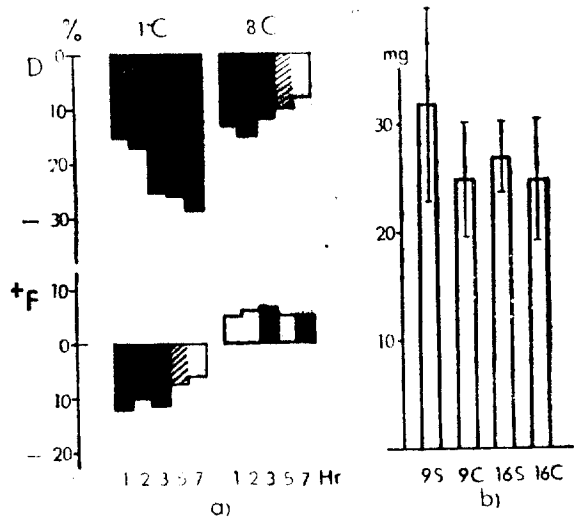
	Hours	1		2		3		5		7		*
	Treatment	$\bar{x}$	±e	$\bar{x}$	±e	$\bar{x}$	±e	$\bar{x}$	±e	$\bar{x}$	±e	
DEXTRAN	Controls single dose	45.7	±1.3	44.3	±0.9	42.6	±1.7	40.5	±1.6	31.5	±1.8	10
	Caffeine 1 × 100 mg/kg or.	38.8	±1.0	36.6	±1.3	31.9	±1.4	30.1	±1.1	24.5	±1.6	10
	Controls repeated dosage	46.8	±1.4	44.4	±1.2	41.5	±1.1	39.0	±0,7	32.4	±1.1	10
	Caffeine 8 × 100 mg/kg or.	40.5	±2.2	37.7	±2.0	36.5	±1.3	35.1	±1.4	29.8	±1.4	10
FORMALDEHYDE	Controls single dose	41.0	±1.3	46.3	±1.4	51.8	±1.1	54.1	±1.6	56.3	±1.5	10
	Caffeine 1 × 100 mg/kg or.	35.9	±0.9	41.5	±1.3	45.9	±1.3	50.3	±1.0	52.9	±1.0	10
	Controls repeated dosage	39.3	±1.6	44.7	±1.2	47.6	±0.7	52.4	±1.1	53.4	±0.8	10
	Caffeine 8 × 100 mg/kg or.	41.2	±1.9	47.3	±1.4	50.8	±0.9	55.2	±1.0	56.2	±0.8	10

* = No. of animals

Table 3. Effect of single and repeated oral administration of caffeine on dextran and formaldehyde oedemas. - $\bar{x}$ = average size of oedema in 1/10 mm, ±e = standard error.

V. Effect of repeated administration of caffeine on the weight of granular tissue formed around cotton pellets

Cotton pellets were inserted under the dorsal skin of the rats used in the preceding experiment on the fifth day after inducing the oedema, the rats being continuously administered 100 mg/kg of caffeine per day. This administration was discontinued the ninth day, the animals were sacrificied by ether inhalation the twelfth day, cotton pellets together with granular tissue dissected, dried at



Graph 5. Effect of single and repeated oral administration of 100 mg/kg of caffeine on the development of experimental oedemas in the hind rat paws (a) and on dry weight of granuloma formation around cotton pellets in mg (b). - a: D = dextran oedema, F = formaldehyde oedema, 1C = a single dose of caffeine, 8C = eight days'application of caffeine. Black columns = $p < 0.02$, shaded columns = $p < 0.05$, white columns = $p > 0.05$. – % = improvement, + % = deterioration of oedema. b: 9S = 9 days'application of saline (including 4 days before the implantation of cotton pellets), 9C = analogous application of caffeine, 16S = 16 days' application of saline (including 11 days before the implantation of cotton pellets), 16C = analogous application of caffeine. All groups consist of 10 rats with the exception of the group 9C including 9 rats. Confidence intervals for P = 95 are given in this graph.

a temperature of 55 °C, and weighed after 11 days. It may be seen from Graph 5b that repeated administration of caffeine did not influence granuloma formation around cotton pellets expressed in dry weight of granular tissue (after substracting 20 mg of cotton).

DISCUSSION

The results of our experiments offer the following conclusion that the effect of caffeine given orally is analogous to the effect of subcutaneous administration, being dependent on the dose used. Caffeine suppressed to the greatest extent

oedemas provoked by egg white and dextran, less influenced were oedemas induced by kaolin and carrageenin, and to the least extent, however still significantly, formaldehyde-induced oedemas.

It is of interest that caffeine suppressed carrageenin-induced abscess even after 24 hours. Unlike the original method (Benitz and Hall 1963) in our experiments caffeine was injected in a single dose at the beginning of the experiment, not in divided doses at the beginning and after 6 hours. This may throw some light on the fact that caffeine given subcutaneously was effective only at the highest dose level, or, when the oral route was chosen, in which cases the resorbtion is obviously slower and the excretion of caffeine from the organism requires a longer time.

A 200 mg dose of caffeine per kg body weight was already toxic, which resulted in deaths of one from ten animals after 24 hours. No changes, however, could have been grossly recognized on the animals, excepting more apparent vasodilatation of the paws with formaldehyde-induced oedemas. However, despite of this profound vasodilatation in the periphery 200 mg/kg of caffeine suppressed the oedema to the greatest extent.

Of some interest is also the finding that repeated pretreatment of rats with caffeine decreased anti-inflammatory effect of caffeine on experimental oedemas, especially on formaldehyde-induced oedema, the development of which was in some phases even significantly deteriorated. On the contrary, this unfavourable effect of long term pretreatment with caffeine did not appear on other non-exudative models of inflammation, e. g. granuloma formation around cotton pellets.

Also these results, however, are in good agreement with our opinion on the role of catecholamines in effecting the exudative phase of experimental oedemas in rats. Our explanation of the effect of caffeine on experimental oedemas is based on the fact that the inhibitory effect of caffeine is due to catecholamines released from the adrenal medulla, (Schaepdryver 1959), though this effect may be brought about also by some other factors. As it may be seen from our previous reports the effect of caffeine on experimental oedemas disappears or is substantially reduced in adrenalectomized rats (Lenfeld, Kroutil 1961) and in adrenal demedullated rats (Lenfeld, Jezdinský, Dušek 1965), and in rats pretreated with DH-ergotoxine and TEAB (Lenfeld, Kroutil 1961). Its effect was not, however, too much influenced by hypophysectomy (Lenfeld, Stojan 1960) and the 11-hydroxysterols level in the urine after repeated administration of caffeine was not significantly increased (Marek, Lenfeld, Jezdinský 1961). Antiserotonin effect of caffeine and its local effect in the rat hind paw was found negligible (Lenfeld, Sládková, Grundman 1963).

This conception could thus explain decreased effect of repeatedly administered caffeine on dextran and formaldehyde oedemas as the depletion of catecholamines from the suprarenal gland which weakens the role of catecholamines in suppressing experimental oedemas.

An analysis of the presented results from the point of view of tolerance and habit towards caffeine or with respect to its diuretic effect and the question of changes in permeability would require more detailed study.

Further, it is obvious from our experiments that anti-exudative effect of caffeine on experimental odemas is not due only to counterirritation in site of subcutaneous injection of the drug because this effect is manifest, though in some cases less

pronounced, also after oral administration which is considered to be less stressing application of the drug to the organism (e. g. Benitz and Hall 1963).

In our further study the effect of caffeine as contained in coffees is examined (Marek 1967).

Acknowledgments. We would like to express our thanks to Marine Colloids, Inc. Springfield, N. J., USA for the carrageenin used in our experiments.

SUMMARY

In the presented study the inhibitory effect of a single oral dose of 100 mg/kg of caffeine has been proved not only on experimental oedemas induced by dextran, formaldehyde and egg white, but also on those provoked by kaolin and carrageenin and on the weight of carrageenin-induced abscess in rats. This inhibitory effect of caffeine given both subcutaneously as well as orally correlates to the dose administered as it has been proved in our experiments on formaldehyde and dextran oedemas and on carrageenin-induced abscess. Long term pretreatment of rats with caffeine reduces its inhibitory effect and leads in formaldehyde-induced oedema even to the deterioration of its development. Repeated administration of caffeine did not effect the weight of granuloma formation around cotton pellets.

О ДЕЙСТВИИ КОФЕИНА НА НЕКОТОРЫЕ МОДЕЛИ ВОСПАЛЕНИЯ У КРЫС

Резюме

Эксперименты показали тормозящее действие однократного введения кофеина через рот в дозе 100 мг/кг на экспериментальные отоки воспроизведенные на лапках крыс не только декстраном, белком и формальдегидом, но тоже каррагинином и каолином и на вес каррагининового абсцесса. Этот эффект исследован на декстрановом и формалиновом отеках и на каррагининовом абсцессе после разных способов введения и разных дозах и обнаружено, что при подкожном введении и введении через рот этот тормозящий эффект кофеина проявляется в зависимости на дозе. При многократном введении кофеина его действие становится ослабленным и воспалительный процесс формалиного отека даже ухудшается. На вес ватовых гранулем многократное введение кофеина не оказало в этих условиях влияния.

PŘÍSPĚVEK K ÚČINKU KOFEINU NA NĚKTERÉ MODELY ZÁNĚTU U KRYS

Souhrn

Pokusy prokázaly tlumivý účinek jednorázové perorální dávky 100 mg/kg váhy zvířete kofeinu nejen na dextranový, bílkový a formalinový, ale i na carrageeninový a kaolinový otok a na váhu carrageeninového abscesu u krys. Na dextranovém a formalinovém otoku a na carrageeninovém abscesu byl sledován účinek po různém způsobu aplikace a v různých dávkách, při čemž bylo zjištěno, že po podkožním i perorálním podání se projevuje tento příznivý účinek kofeinu v závislosti na dávce. Při dlouhodobějším užívání kofeinu dochází k oslabení jeho účinků a na formalinovém otoku dokonce ke zhoršování jeho průběhu. Na váhu vatových smotků nemělo opakované podávání kofeinu za těchto podmínek žádný vliv.

REFERENCES

1. *Benitz K. F., Hall L. M.:* Arch. int. Pharmacodyn. *144*, 185 (1963).
2. *Kroutil M.:* Acta Univ. Olomuc. *35*, 19 (1964).
3. *Lenfeld J.:* Čs. fysiol. *7*, 331 (1958).
4. *Lenfeld J., Jezdinský J., Dušek J.:* Čs. fysiol. *14*, 301 (1965).
5. *Lenfeld J., Kroutil M.:* Farmakoterapie zánětů. Praha, SZdN, 1961.
6. *Lenfeld J., Sládková O., Grundman M.:* Čas. lék. čes. *102*, 554 (1963).
7. *Lenfeld J., Stojan B.:* Čs. fysiol. *9*, 505 (1960).
8. *Lenfeld J., Stojan B.:* Arch. exp. Path. Pharmak. *241*, 162 (1961).
9. *Marek J.:* Acta Univ. Olomuc. *44*, 113 (1967).
10. *Marek J., Lenfeld J., Jezdinský J.:* Acta Univ. Olomuc. *23*, 155 (1961).
11. *Meier R., Schuler W., Desaulles P.:* Experientia (Basel) *6*, 469 (1950).
12. *de Schaepdryver A. F.:* Arch. int. Pharmacodyn. *121*, 222 (1959).

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A COMPARISON OF THE RELATIVE TOXIC, EMETIC AND CONVULSIVE ACTIONS OF A SERIES OF METHYLATED XANTHINE DERIVATIVES

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Several methylated xanthines are presently employed therapeutically as diuretics for the treatment of edema (Boyer, 1943; Fowell *et al.*, 1949). They are widely used also as bronchodilators (Lamson and Bacon, 1941; Bresnick *et al.*, 1949). While such drugs are of value, they produce several undesirable side-effects, i.e. nausea, emesis, wakefulness and convulsions, which detract from their usefulness.

We have been concerned with the comparison of a series of 1 and 7 substituted theobromine and theophylline derivatives with those xanthines presently in use as diuretics and bronchodilators. It was considered that a drug retaining the diuretic and bronchodilator activities of theophylline and theobromine but lacking some of the toxic, convulsive and emetic properties would be desirable.

This paper deals with the acute toxic, the relative emetic and the convulsive properties of the same series of methylated xanthine congeners which have been compared for their diuretic (McColl *et al.*, 1953) and for their bronchodilator properties.

METHODS. Acute toxicity was determined for: theophylline; theophylline ethylenediamine (aminophylline); 7 butyl, 7-isoamyl, 7-allyl, 7-benzyl, 7-acetic acid, 7-hydroxyethyl, 7-β γ-dihydroxypropyl, 7-chloroethyl, 7-diethylaminoethyl, and 7-β-methoxy-γ-hydroxypropyl theophylline; theobromine sodium acetate; 1-butyl, 1-isoamyl and 1-allyl theobromine; and caffeine sodium benzoate.

The toxicity was estimated in white mice (18-22 gm.) of both sexes by administering the compound intraperitoneally and orally. There were six animals at each dose level, and three to five dose levels were used for each drug. Eighteen to thirty animals were thus required for the estimation of each LD_{50}. These data, expressed as per cent mortality, were used in calculating the LD_{50} by the probit method of Finney together with the 95 per cent confidence limits (Burn *et al.*, 1950).

These animals were kept under observation, at a constant temperature of 25°C, for one week following the administration of the xanthine. Deaths which occurred within that interval were included in the mortality data from which the LD_{50} was calculated. With the exception of 7-acetic acid theophylline and 7-benzyl theophylline, all deaths occurred within 24 hours.

Whenever a solvent other than water was used to dissolve a particular xanthine, it was tested alone for its toxicity in the concentration used with the xanthine. These included 25 per cent propylene glycol corn oil, sodium acetate, and sodium benzoate solutions. In no case did these substances in the amounts used, demonstrate any toxicity in mice.

Convulsive Actions. Coincident to the administration of the xanthines for the determina-

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tion of acute toxicity, observations were made regarding stimulation of the central nervous system as manifest by convulsions.

Relative Emetic Activity. The emetic activity for each of the xanthines was compared in dogs and cats. One-half the dose determined to be the oral LD_{50} (mgm./kgm.) for the mouse was administered to these animals. This dose was reduced by intervals until no emesis was observed. The figure reported (ED_{50}) was that dose which was estimated to produce emesis in 50 per cent of the animals. The drug was administered orally in gelatin capsules with a small amount of methylene blue to aid in the observation of emesis.

A placebo, of lactose and methylene blue, was randomly administered during the course of these experiments. This was done to ascertain if (a) a conditioned reflex of emesis was being established and (b) if the methylene blue "tracer" was causing emesis. There was no evidence that either condition complicated this test of the relative emetic activity of these xanthines.

RESULTS. Table 1 shows the intraperitoneal LD_{50} values in the mouse. Included also are the 95 per cent fiducial limits for each LD_{50} and the slopes of the regression line for the log-dose mortality curve. The drugs are arranged in order of their decreasing toxicity.

LD_{50} values ranged from 100 to 1800 mgm./kgm. The most toxic of these xanthines was 1-allyl theobromine with an LD_{50} of 102 mgm./kgm. Theophylline, 7-isoamyl theophylline, 1-isoamyl theobromine, 7-benzyl theophylline, 1-butyl theobromine, 7-diethylaminoethyl theophylline, aminophylline, 7-butyl theophylline, 7-chloroethyl theophylline and 7-allyl theophylline had LD_{50}'s from 200–300 mgm./kgm. Caffeine sodium benzoate, 7-hydroxyethyl theophylline and theobromine sodium acetate were less toxic. The least toxic of these

TABLE 1

Acute intraperitoneal toxicity in mice

Xanthine	LD_{50}	95% Fiducial Limits	Slope
	mgm./kgm.	*mgm./kgm.*	
1-Allyl theobromine	102	79–130	6.5
7-Isoamyl theophylline†	211	203–221	6.9
Theophylline	217	208–225	34.7
1-Isoamyl theobromine†	222	203–243	8.9
7-Benzyl theophylline*	228	199–319	13.7
1-Butyl theobromine†	230	204–260	11.9
7-Diethylaminoethyl theophylline	254	234–275	18.9
Aminophylline	257	247–268	15.9
7-Butyl theophylline†	272	236–319	8.9
7-Chloroethyl theophylline	281	264–299	15.4
7-Allyl theophylline	299	273–327	16.1
Caffeine and sodium benzoate	525	481–574	17.5
7-Hydroxyethyl theophylline	599	527–681	11.3
Theobromine sodium acetate	789	712–874	14.0
7-Acetic acid theophylline‡	1180	903–1565	5.1
7-β-Methoxy-γ-hydroxypropyl theophylline	1488	1203–1840	7.0
7-β-γ-Dihydroxypropyl theophylline	1770	1750–1807	8.2

* In corn oil.

† In 25 per cent propylene glycol solution.

‡ In 0.2 *M* sodium acetate (pH 4.6) solution.

TABLE 2

Acute oral toxicity in mice

Xanthine	LD_{50}	95% Fiducial Limits	Slope
	mgm./kgm.	*mgm./kgm.*	
1-Allyl theobromine	191	177–207	18.8
7-Allyl theophylline	315	279–356	11.4
Theophylline	332	270–408	14.0
7-Chloroethyl theophylline	374	343–381	18.4
Theophylline ethylenediamine	391	276–441	4.4
7-Butyl theophylline*	617	427–708	7.7
1-Butyl theobromine*	667	632–695	26.8
7-Isoamyl theophylline*	723	504–1038	4.9
1-Isoamyl theobromine*	772	694–326	13.3
7-Hydroxyethyl theophylline	855	823–887	3.6
Caffeine and sodium benzoate	878	736–1047	8.8
7-Diethylaminoethyl theophylline	1237	1139–1344	17.8
Theobromine sodium acetate	1356	1124–1862	6.2
7-β-Methoxy-γ-hydroxypropyl theophylline	1681	1490–1895	11.2
7-β-γ-Dihydroxypropyl theophylline	1954	1497–2251	5.9
7-Acetic acid theophylline†	2733	1733–4310	2.2

* In 25 per cent propylene glycol solution.

† In 0.2 *M* sodium acetate (pH 4.6) solution.

xanthine derivatives were 7-acetic acid theophylline, 7-β-methoxy-γ-hydroxypropyl theophylline and 7-β-γ-dihydroxypropyl theophylline.

Table 2 shows the data for the acute oral toxicity in mice of the same compounds. The drugs are again arranged in order of their decreasing toxicity.

The 1-allyl theobromine derivative was again observed to be the most toxic. The 7-acetic acid theophylline was the least toxic of these compounds. However, the latter xanthine was the most variable in action, and the figures represent the toxicity observed over a one-week period.

With the exception of 7-β-methoxy-γ-hydroxypropyl theophylline, the lethal dose was generally larger by the oral route than by the intraperitoneal route. For this compound the amounts fell within the range of 95 per cent confidence limits.

In most cases the difference between the oral LD_{50} and intraperitoneal LD_{50} was no greater than a factor of two. However, with 7-diethylaminoethyl theophylline, the oral LD_{50} was five times greater than the intraperitoneal LD_{50}. A similar situation was encountered with the 1- and 7-isoamyl derivatives and the 1- and 7-butyl derivatives, although in these latter instances the difference was of the order of three.

Comparative Emetic Action of the Xanthine Derivatives: Table 3 summarizes the estimated dose of each xanthine observed to produce emesis in the cat and in the dog. The figure for the ED_{50} (emetic dose) shown (columns II and III) is that dose required to produce emesis in 50 per cent of the animals. This can be considered only as approximate due to the small number of animals tested. For purposes of [illegible] the oral LD_{50} in t[illegible]

TABLE 3

Comparative emetic actions of xanthines in dogs and cats

Xanthine	I Oral Mouse LD_{50}	II Dog ED_{50}	III Cat ED_{50}	IV Dog Emetic Index	V Cat Emetic Index
		mgm./kgm.	*mgm./kgm.*		
7-Hydroxyethyl theophylline	855	380	110	0.45	0.13
1-Allyl theobromine	191	73	—	0.38	—
8-Chlorotheophylline	440*	165	82	0.38	0.19
Theophylline	332	120	120	0.36	0.36
Theophylline ethylenediamine	391	140	100	0.36	0.26
7-Butyl theophylline	617	210	60	0.34	0.10
7-Chloroethyl theophylline	374	112	70	0.30	0.19
7-Allyl theophylline	315	80	80	0.25	0.25
Theobromine sodium acetate	1356	338	—	0.25	—
Caffeine and sodium benzoate	878	215	—	0.25	—
7-Diethylaminoethyl theophylline	1237	300	210	0.24	0.18
7-β-Methoxy-γ-hydroxypropyl theophylline	1681	400	300	0.24	0.18
7-β-γ-Dihydroxypropyl theophylline	1954	375–750	94–187	0.2–0.3	0.05–0.1
7-Isoamyl theophylline	723	105	80	0.15	0.11
1-Isoamyl theobromine	772	100	100	0.13	0.13
7-Acetic acid theophylline	2733	350	300	0.13	0.11
1-Butyl theobromine	667	43	43	0.06	0.06

* LD_{50} value taken from DeJongh *et al.* (1951).

(column I). The figures in columns IV and V result from the ratio: ED_{50} Dog or Cat/oral LD_{50} (mouse). This ratio is such that the smaller the value, the greater the emetic action of the compound relative to the lethal action in the mouse. It is emphasized that this ratio is formed from data from different species. Its value depends on the assumption that the oral LD_{50} for the dog or cat would be of a similar order as for the mouse. This may not be true.

From the data in table 3, it may be observed that the ED_{50} appears to be generally of the same order between species. Exceptions are observed with 7-β-γ-dihydroxypropyl theophylline, 7-butyl theophylline and 7-hydroxyethyl theophylline. With these derivatives, the dose causing emesis in the cat was one-half to one-third that for the dog.

In the dog, 7-hydroxyethyl theophylline, 1-allyl theobromine, 7-β-γ-dihydroxypropyl theophylline and 7-butyl theophylline were the best tolerated of the xanthines tested, and in that order of increasing emetic action. The most poorly tolerated derivatives included 1-butyl theobromine, 1-isoamyl theobromine, 7-acetic acid theophylline and 7-isoamyl theophylline. The other xanthines comprised a group somewhat intermediate in their emetic action.

In the cat, theophylline and aminophylline were the best tolerated of the xanthines. The 1-butyl theobromine, 7-butyl theophylline, 7-β-γ-dihydroxypropyl theophylline, 7-acetic acid theophylline, 7-isoamyl theophylline, 1-isoamyl theobromine, and 7 hydroxyethyl theophylline derivatives were the most poorly tolerated in the species.

For both species, 1-butyl, 1-isoamyl, 7-acetic acid and 7-isoamyl derivatives were the most active in causing emesis. 7-Diethylaminoethyl theophylline was not well tolerated, and fatalities were observed at the doses employed.

Theophylline and aminophylline were both well tolerated in the dog and in the cat, as was 1-allyl theobromine in the dog.

The emetic action of aminophylline (750 mgm./kgm.) and 7-β-γ-dihydroxypropyl theophylline (750 mgm./kgm.) was also observed in the dog following intramuscular injections. In both cases the animals salivated profusely, vomited and died within 15 to 24 hours. Post-mortem examination revealed the stomach distended with fluid. Petechial haemorrhages were observed throughout the gastric mucosa of the animals that received the 7-β-γ-dihydroxypropyl derivative.

Convulsant Actions: Observations of the occurrence of central nervous system stimulation as manifest by convulsions in the mouse by these derivatives were made coincident to the determination of the oral and the intraperitoneal LD_{50} determinations. The 7-diethylaminoethyl produced the most violent convulsions of any of the xanthines tested. Convulsions were observed in the case of theophylline, aminophylline, caffeine, 7-isoamyl theophylline, 7-butyl theophylline, 1-butyl theobromine and 7-chloroethyl theophylline.

Discussion. There are few reports in the literature in which the acute toxicities of methylated xanthines have been estimated in the same species and by the same routes as the present work. The intraperitoneal route was chosen for the estimation of the toxicity as it approximates the intravenous route and avoids the arbitrary rate of injection which tends to complicate the latter.

It is difficult to compare many of the previous reports as they are concerned with the absolute rather than the relative toxicity. Allmark (1951) has described the difficulties of such absolute comparisons. This work represents the relative toxicity of a series of compounds determined in a comparable population and under the same environment.

Our data agree well with those reported by Jensen (1949), Thompson and Warren (1946) and Hildebrandt (1952) for aminophylline. The same agreement is noted for theophylline as reported by Jensen (1949) and Hildebrandt (1952).

For the acute toxicity of 7-β-γ-dihydroxypropyl theophylline, our figures for the LD_{50} fall within the fiducial limits of that reported by Jensen (1949). The toxicity of 7-chloroethyl theophylline relative to aminophylline was found to be similar to that observed by Blumberg *et al.* (1953).

Scott, Anderson and Chen (1946) reported on the toxicity by the intravenous route of a series of theobromine derivatives including 1-allyl, 1-butyl, and 1-isoamyl. While the figures for the LD_{50} showed a greater toxicity due to the different routes of administration, it is interesting to note the agreement in the relative order of toxicity of these three derivatives.

Acute toxic reactions to theophylline derivatives have been recorded in the human. Allard (1905) reported two deaths following theophylline administration. Merrill (1943) described five deaths following aminophylline. Bresnick, Woodar and Sageman (1948) also reported three deaths after intravenous ad-

ministration of aminophylline to cardiac patients. The mechanism of death in these cases is unknown.

While these untoward deaths have been recorded, greater limitations to the use of these drugs are due to undesirable side effects such as central excitation, nausea and vomiting. The di- and tri-methylated xanthines have been postulated as causing nausea and emesis by direct gastric irritation. Brown and Riseman (1937) observed an increase in the occurrence of gastric distress with an increasing dose of theophylline salts. Bliss and Morrison (1933) reported that the various salts of theophylline and theobromine investigated by them produced renal and gastro-intestinal irritation at practically the same dose level.

Luduena (1942) concluded from his studies that 0.2–0.48 gm. of intravenous aminophylline two to three times daily did not appear to be harmful to asthmatic patients. He also observed that 7 mgm./kgm./day intravenously in rabbits did not produce any histological evidence of tissue damage.

There is evidence that the emetic action is caused not only by a local gastric reflex but also by an extra-gastric component. Allard (1905) observed vomiting in cats following the subcutaneous administration of aminophylline, as did Scott *et al.* (1946) following intravenous injection. We have obtained emesis following intramuscular injections with both aminophylline and 7-β-γ-dihydroxypropyl theophylline.

There are few data available on the relative incidence of emesis with other than the salts of the methylated xanthines. Scott *et al.* (1946) investigated a series of theobromine derivatives (including isoamyl, allyl and butyl) and recorded the number of dogs which vomited following an intravenous dose of 10 mgm./kgm. At this dose, caffeine and theobromine caused no emesis; 1-butyl theobromine caused emesis in two out of seven, while 1-isoamyl theobromine caused emesis in one out of seven. In the human they reported nausea in 50 per cent of the subjects following 200 mgm. of 1-allyl theobromine and emesis in one person out of six. Theophylline and caffeine did not produce nausea or vomiting at a similar dose.

Kattus, Newman and Franklin (1951) studied the emetic and diuretic activities of a series of 1 and 1, 3 substituted xanthines in the dog. They concluded that the diuretic activity appeared to be paralleled by the emetic activity.

Our data do not suggest this relationship of the emesis in dogs and cats to the diuretic action in the rat (McColl *et al.*, 1953). The most effective diuretics in the rat (7-chloroethyl theophylline, theophylline and aminophylline) were among those best tolerated on the basis of the emetic ratio in cats and in dogs.

Blumberg *et al.* (1953) reported that 7-chloroethyl theophylline was less irritating to the gastro-intestinal tract than was aminophylline. Our data showed that these derivatives had about the same emetic activity in the dog.

Luermann and Boeres (1952) observed that 7-hydroxyethyl theophylline was no more effective than other theophylline compounds, but was better tolerated. Our results would appear to agree with this in the dog, but not in the cat, when compared with aminophylline.

Emesis can be produced with methylated xanthines following either oral or

parenteral administration. In the first instance, it may be a local reflex while in the second, it appears to be an extra-gastric reflex and perhaps a central one. It would appear that emetic properties were common to all members of the groups studied. The fact that the emetic ratios for several drugs were similar suggests that the emetic properties are proportionate to oral toxicity.

Central excitation and convulsions are drawbacks to the use of methylated xanthines as bronchodilators or as diuretics. The wakefulness produced by caffeine may have led earlier investigators to try theophylline for relief of asthma. Allard (1905) described convulsions as part of the terminal picture in his fatal cases.

In comparing the action of the series of theobromine derivatives they investigated, Scott *et al.* (1946) did not observe convulsions with 1-butyl theobromine or 1-isoamyl theobromine. Also, Blumberg *et al.* (1953) reported no convulsions with 7-chloroethyl theophylline. These variations from the present work may be due to difference in routes of administration and in doses.

Mercier, Mercier and Sester (1949) studied the analeptic and convulsive actions of several xanthine derivatives in the dog. They observed convulsions with these compounds in the following order of decreasing effect: 7-diethylaminoethyl theophylline, theophylline and 7-hydroxyethyl theophylline.

SUMMARY

The acute intraperitoneal and oral toxicity in terms of LD_{50} mgm./kgm. was determined in the mouse for a series of 1 and 7 substituted xanthines.

The values calculated for the LD_{50} by the intraperitoneal route in terms of mgm./kgm. varied from 100 to 1800. 1-Allyl theobromine was the most toxic derivative while 7-β-γ-dihydroxypropyl theophylline was the least toxic.

By the oral route, the values for the LD_{50} in terms of mgm./kgm. varied from 190 to 2700. Again 1-allyl theobromine was the most toxic while 7-acetic acid theophylline was the least toxic.

7-Diethylaminoethyl theophylline was the most active derivative in producing convulsions in the mouse. Convulsions were also observed with 7-isoamyl theophylline, 7-butyl theophylline, caffeine, theophylline, aminophylline, 1-butyl theobromine and 7-chloroethyl theophylline.

By oral administration theophylline and aminophylline were well tolerated in both the dog and the cat. In both species, 1-butyl, 1-isoamyl theobromine, 7-acetic acid and 7-isoamyl theophylline were the most active of these derivatives in producing emesis. 7-Diethylaminoethyl theophylline caused fatalities in both the dog and the cat.

REFERENCES

Allard, E.: Deutsch. Arch. klin. Med., **80**: 510, 1905.

Allmark, M. G.: J. Am. Pharm. A. (Scient. Ed.), **40**: 27, 1951.

Bliss, A. R., and Morrison, R. W.: J. Lab. & Clin. Med., **19**: 245, 1933.

Blumberg, H. C., Stein, E., Schlesinger, A. M., and Gordon, S. M.: Fed. Proc., **12**: 409, 1953.

Boyer, N. H.: J.A.M.A., **122**: 306, 1943.

Bresnick, E., Beakey, J. F., Levenson, L., and Segal, M. S.: J. Clin. Invest., **28:** 1182 1949.
Bresnick, E., Woodar, W. K., and Sageman, C. B.: J.A.M.A.; **136:** 397, 1948.
Brown, M. G., and Riseman, J.E.F.: J.A.M.A.; **109:** 256, 1937.
Burn, J. H., Finney, D. J., and Goodwin, L. G.: Biological Standardization, 2nd. Ed., Oxford University Press, 1950.
DeJongh, D. K., VanProosdij-Hartzema, E. G., and Knoppers, A. T.: Acta Physiol. et. pharmacol. neerl., **2:** 63, 1951.
Fowell, D. M., Winslow, J. A., Sydenstricker, V. P., and Wheeler, N. C.: Arch. Int. Med., **83:** 150, 1949.
Hildebrandt, F.: Deutsche med. Wchnschr., **77:** 13, 1952.
Jensen, K. B.: Arch. Pharm. Chem., **56:** 741, 1949.
Kattus, A. A., Newman, E. V., and Franklin, J.: Bull. Johns Hopkins Hosp., **89:** 1, 1951.
Lamson, R. W., and Bacon, L. C.: J.A.M.A., **116:** 915, 1941.
Luduena, F. P.: This Journal, **75:** 316, 1942.
Luermann, O. W., and Boares, W.: Deutsch. med. Wchnschr., **77:** 15, 1952.
McColl, J. D., Parker, J. M., and Ferguson, J. K. W.: This Journal, **110:** 36, 1953.
Mercier, F., Mercier, J., and Sestier, M. R.: Tr. Soc. Pharm. Montpellier, **7:** 14, 1949.
Merrill, G. A.: J.A.M.A., **123:** 1115, 1943.
Scott, C. C., Anderson, R. C., and Chen, K. K.: This Journal, **86:** 113, 1946.
Thompson, C. R., and Warren, M. R.: J. Lab. & Clin. Med., **31:** 1337, 1946.

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THE EFFECT OF CAFFEINE ON DRUG METABOLISM

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THE hepatic drug-metabolizing enzyme system is stimulated by a variety of compounds.[1] Hence it is not surprising that many compounds to which we are exposed daily can affect the enzyme system. For instance, constituents of cigarette smoke such as nicotine,[2] 3-methylcholanthrene, and 3,4-benzpyrene[1] are known to stimulate drug metabolism by the hepatic microsomal system. Caffeine is another compound that is commonly ingested as a constituent of coffee, tea and cocoa.

In the present investigation, the effect of caffeine on drug metabolism was examined. The data presented indicate that caffeine acts as an inducer of the microsomal system.

Experimental

Preparation of hepatic microsomal system: Rats were killed either by cervical dislocation or by decapitation. The livers were removed immediately and homogenized with an all-glass Potter homogenizer. Homogenates were routinely prepared using three volumes of 0.1 M, pH 7.4, potassium phosphate buffer. A supernatant fraction containing the microsomes was prepared by centrifuging the homogenate in a Servall refrigerated centrifuge for 10 min at 10,000 x g.

Enzymic assays: To assay the drug-metabolizing activity, 1 ml of the post-mitochondrial supernatant fraction (25-35 mg protein) was mixed with a solution containing tris buffer, pH 8.0 (200 μmoles), glucose-6-phosphate (25 μmoles), nicotinamide (15 μmoles), $MgCl_2$ (25 μmoles), NADP (0.25 μmole), NAD (5 μmoles) and various substrates in a final volume of 3.1 to 3.3 ml. Incubation was carried out in a Dubnoff metabolic shaker for 30 min at 37°C in air.

The metabolic pathways, the amounts of substrate per beaker, and the assay methods used were: (a) aromatic ring hydroxylation, 10 μmoles of acetanilide, colorimetric method of Ninomiya[3] for p-hydroxyacetanilide; and (b) O-demethylation, 7 μmoles of o-nitroanisole, spectrophotometric determination of o-nitrophenol.[4]

Protein concentration was determined by the method of Lowry et al.[5] The enzymic activities were expressed as μmoles of product formed per g of protein in 30 min. The data were analyzed by Students' "t" test.

Results

To examine the effect of caffeine on the microsomal system, rats were orally administered twice daily with 100, 75, or 50 mg/kg for three days. They were sacrificed at least 4 hr after the last injection. In all cases the microsomal activities with respect to acetanilide, o-nitroanisole, and aminopyrine metabolism were higher in the caffeine-treated groups than in the control group. The data presented in Table 1 are on rats treated twice daily with 30 mg/kg of caffeine.

TABLE 1

Effect of caffeine on hepatic microsomal system

Groups	μMoles of products formed per g of liver protein	
	Acetanilide	o-Nitroanisole
Control	7.3 ± 1.6(4)	7.8 ± 1.6(8)
Caffeine-treated	15.6 ± 3.5(4)	10.2 ± 2.3(9)
	$p < .005$	$p < .025$

Male Sprague-Dawley rats weighing 190-200 g were orally administered with 30 mg/kg of caffeine twice daily for three days. Figures in parentheses refer to the number of rats in each group. Results are expressed as the mean ± S.D.

Since caffeine had been reported[6] to stimulate the formation of 1-naphthol from naphthalene *in vitro*, the effect of preincubating the microsomal system with 1 to 5 x 10^{-4} M caffeine before the addition of acetanilide was examined. The formation of p-hydroxyacetanilide was not stimulated in the *in vitro* system. Also, examination of the kinetics of acetanilide hydroxylation indicated that the microsomal system prepared from caffeine-treated rats showed essentially the same affinity constant to acetanilide as that from control rats. The Km values, obtained directly from Lineweaver-Burk plot (Fig. 1), were 9.1 x 10^{-4} M for the control and 7.7 x 10^{-4} M for the caffeine-treated microsomal system.

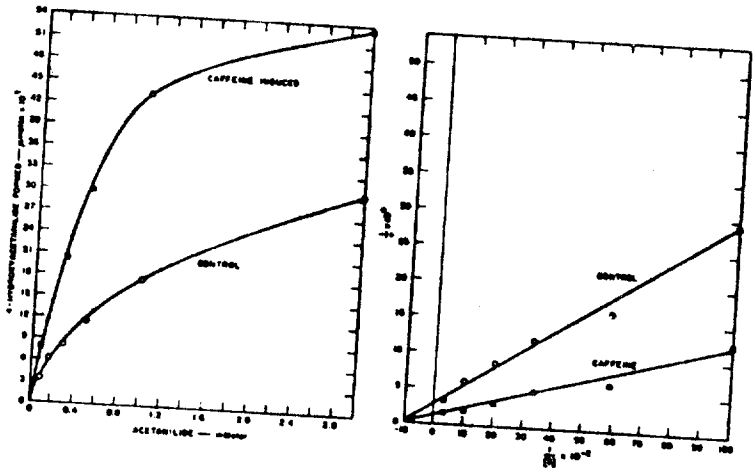

FIG. 1

Effect of acetanilide concentration on the rate of hydroxylation reaction.

To investigate the possible role of caffeine as a microsome inducer, the effect of inhibitors of protein synthesis on the stimulatory action of caffeine was studied. Ethionine and puromycin have been shown to counteract the increase in microsomal activity due to microsome inducers such as phenobarbital and 3-methylcholanthrene.[7] As shown by the data presented in Table 2, the amount of ethionine used considerably decreased the caffeine effect. Apparently, an insufficient amount of ethionine was used since the induction effect due to

TABLE 2

Effects of ethionine and puromycin on the stimulatory effect of caffeine on the microsomal system.

Experiment no.	Treatment	μMoles of p-hydroxyacetanilide formed per g of liver protein	
1	None	9.6 ± 1.5	
	Caffeine	22.2 ± 3.6[a]	$p < .01$
	Caffeine + Ethionine	14.6 ± 3.4[a]	$p < .01$
	Phenobarbital + Ethionine	13.7 ± 5.1	$p < .01$
2	None	6.4 ± 0.6	
	Caffeine	9.5 ± 2.1	$p < .05$
	Caffeine + Puromycin	5.7 ± 1.2	$p > .05$

[a]Significantly different from each other, $p < .01$. In Experiment 1, six rats weighing 170 g were used in each group except for the last group (four rats). Caffeine (75 mg/kg, orally) or phenobarbital (38 mg/kg, ip) was administered twice daily for three days. Ethionine (245 mg/kg, ip) was injected twice, 30 min apart, prior to the first daily dose of caffeine or phenobarbital. In Experiment 2, four rats weighing 200 g were used in each group. Puromycin (2.5 mg/injection) was injected ip four times a day for three days during working hours.

phenobarbital also was not completely suppressed. However, these data, together with those from the puromycin experiment, strongly indicate that caffeine is acting as a microsome inducer.

Since theophylline and theobromine are also contained in beverages and are used as drugs, it was of interest to compare these compounds with caffeine for their induction effects. As shown in Table 3, the relative potency of these compounds as microsome inducers in decreasing order was caffeine, theophylline

TABLE 3

Relative potency of caffeine, theophylline and theobromine as microsome inducers.

Compounds	Dosage (mg/kg/day)	μMoles of p-hydroxyacetanilide formed per g of liver protein
None		7.6 ± 1.5 (5)
Caffeine		16.0 ± 2.9[a] (5)
Theophylline	150	14.0 ± 4.9[a] (5)
Theobromine		11.9 ± 3.0[a] (5)
None		7.2 ± 1.1 (6)
Caffeine		14.3 ± 3.2[a] (6)
Theophylline	75	12.0 ± 3.3[a] (6)
Theobromine		8.8 ± 2.0 (6)
None		7.3 ± 1.2 (5)
Caffeine		11.5 ± 3.6[a] (4)
Theophylline	40	8.4 ± 1.7 (5)
Theobromine		7.8 ± 1.6 (5)

[a]Significantly different from the control at $p < .05$. Figures in parentheses refer to the number of rats in each group. Male Sprague-Dawley rats weighing approximately 180 g were orally treated with 75, 37.5, or 20 mg/kg of each of the compounds twice daily for three days.

and theobromine. It has been reported[8] that there is no conversion of one of these compounds to the other in man, although the metabolic pathways of these N-methylated xanthines overlap to some extent.

The induction effect discussed heretofore has been with purified caffeine itself. In the data presented in Table 4, it is shown that drinking of coffee

TABLE 4

Effect of coffee and tea on microsomal activity.

Beverage	Concentration of ingredients (g/100 ml)	Average daily consumption		μMoles of p-hydroxyacetanilide/g liver protein	
		Volume (ml)	Caffeine (mg/kg)		
Water	-	143	-	8.3 ± 1.3	
Coffee	2.84	70	76.7	13.9 ± 3.3	$p < .05$
Tea	1.86	69	74.6	14.7 ± 2.2	$p < .01$

Four rats (average weight, 314 g) were used per group. They were allowed to drink the beverages ad libitum for three days. The rats were sacrificed the day after the beverages were removed. Instant Maxwell House coffee (5% caffeine) and Instant Nestea (7.3% caffeine) were used in these experiments. Caffeine was determined by a spectrophotometric method.[9]

or tea equivalent to about 75 mg/kg of caffeine by the rat resulted in elevated activity of the hepatic microsomal system.

Discussion

Data have been presented to show that caffeine as well as coffee and tea increase the drug-metabolizing activity of the liver. This effect is not due to the direct interaction of caffeine with the microsomes but appears to be due to increased enzyme synthesis.

The minimum amount of caffeine intake per day necessary to show this effect was 40 mg/kg (Table 3), which is equivalent to about 35 cups of coffee a day for a 70-kg man on a straight weight basis. However, if based on the metabolic weight* this is equivalent to about 8 cups of coffee for a 70-kg man. In keeping

*The metabolic weight[10] which is expressed as weight$^{3/4}$ is kg of body weight to the 3/4 power. Thus, the metabolic weight of a 0.2-kg rat and a 70-kg man are 0.3 kg and 24.2 kg, respectively.

with this concept a preliminary study indicated that the biological half-life of caffeine in a rat was approximately five hr when 25 mg/kg was injected intraperitoneally. The half-life of caffeine in man was reported to be about 4.5 hr for a dose of 7 mg/kg.[9]

Thus, it is not unlikely, on the basis of data presented above, that coffee or tea drunk moderately by man may have an inducing effect on his liver drug-metabolizing system.

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References

1. A. H. CONNEY, Pharmacol. Rev. 19, 317 (1967).
2. D. G. WENZEL and L. L. BROADIE, Toxicol. Appl. Pharmacol. 8, 455 (1966).
3. T. NINOMIYA, J. Pharmaceut. Soc. Japan 85, 394 (1965).
4. K. J. NETTER, Arch. Exptl. Pathol. Pharmakol. 238, 292 (1960).
5. O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. Biol. Chem. 193, 265 (1951).
6. J. BOOTH and E. BOYLAND, Biochem. J. 70, 681 (1958).
7. A. H. CONNEY and J. J. BURNS, Advan. Enzyme Regulation 1, 189 (1963).
8. H. H. CORNISH and A. A. CHRISTMAN, J. Biol. Chem. 228, 315 (1957).
9. J. AXELROD and J. REICHENTHAL, J. Pharmacol. 107, 519 (1953).
10. M. KLEIBER, The Fire of Life p. 209, John Wiley and Sons, Inc., New York (1961).

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Nature of the Effect of Caffeine on the Drug-Metabolizing Enzymes[1]

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Pretreatment of the rat for 3 days with caffeine resulted in an elevation in the drug-metabolizing activity by the hepatic microsomal system. This effect is counteracted by the simultaneous administration of actinomycin D. Caffeine also shortened the duration of action of zoxazolamine and methyprylon in the rat. The stimulatory effect of caffeine on drug metabolism was additive to that of phenobarbital when acetanilide was the substrate but not when *o*-nitroanisole or aminopyrine was used. Attempts to show increased amino acid incorporation or an increase in cytochrome P-450 in the microsome were not successful. An interesting feature of the stimulatory effect of caffeine on drug metabolism is its short duration after the termination of treatment. Phenobarbital was used throughout this study as a reference compound.

The hepatic drug-metabolizing enzyme system is stimulated by a variety of compounds to which we are constantly exposed. Thus, the metabolism of drugs is accelerated by drugs, insecticides, constituents of cigarette smoke (1), and alcohol (2). More recently, we reported that caffeine administered twice daily to the rat for 3 days stimulated the drug-metabolizing activities (3).

The purpose of the present investigation was to obtain additional evidence for and to investigate the mechanism of stimulation of drug metabolism by caffeine. To this end, the effects of caffeine on the rat liver were compared with those of phenobarbital.

MATERIALS AND METHODS

Male Sprague-Dawley rats purchased from Simonsen Laboratories, Inc., Gilroy, California, were used throughout. They were kept in cages (4–6 rats per group) suspended over Absorb-Dri or San-i-cel and were maintained on a stock diet and water *ad libitum*. The weight of the rats generally ranged from 160 to 300 g (40–60 days old) except for those used in the amino acid-incorporation studies described below.

The rats were generally pretreated with caffeine and phenobarbital for 3 days. The dosage and the route of administration for each experiment are described in the tables. Control rats were always treated with an equivalent volume of the vehicle via the same route. The first dose of caffeine or phenobarbital usually was administered in the afternoon of the first day. The animals were given two doses of caffeine or phenobarbital in the morning and afternoon during the next 2 days and a final dose on the morning of the fourth day. This schedule is referred to as the 3-day pretreatment. The rats were sacrificed in the afternoon of the fourth day either by cervical dislocation or by decapitation. The livers were removed and frozen intact immediately or the 10,000*g* supernatant fraction was prepared and then was frozen for subsequent assays.

The livers were homogenized in 3 ml of cold potassium phosphate buffer (0.1 M), pH 7.4, for each gram of liver. A postmitochondrial supernatant fraction containing the microsomes was prepared by centrifuging the homogenate in a Servall refrigerated centrifuge for 10 min at 10,000*g*.

To assay the drug-metabolizing activity, 1 ml of the supernatant fraction (25–35 mg protein) was mixed with a solution containing Tris buffer, pH 8.0 (200 μmoles), glucose-6-phosphate (40 μmoles), nicotinamide (15 μmoles), $MgCl_2$ (25

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μmoles), NADP (0.25 μmoles), NADH (5 μmoles), and various substrates in a final volume of 3–4 ml. The incubation was carried out in a Dubnoff metabolic shaker for 30 min at 37° in air. All reactions were approximately linear during this period. The amounts of substrates added per incubation beaker were 10 μmoles for acetanilide, *p*-chloro-*N*-methylaniline, or aminopyrine and 7 μmoles for *o*-nitroanisole. The results are expressed as the number of micromoles of product formed ± standard deviation per g of 10,000*g* supernate protein. Comparisons of the mean enzymic activities between the control and experimental rats were made by Student's *t* test.

The assay techniques for drug-metabolizing activities for acetanilide, *o*-nitroanisole, and aminopyrine have been described previously (4, 5). The metabolism of *p*-chloro-*N*-methylaniline was measured by the method of Kupfer and Bruggeman (6). The CO-binding pigment (cytochrome P-450) content of the microsomes was assayed by the method of Schenkman *et al.* (7).

In the incorporation studies of labeled amino acid, rats were killed 90 min after L-phenylalanine-2,3-^{3}H (Amersham-Searle Corp.) was injected. To facilitate handling of large numbers of rats and also to minimize the amount of labeled amino acid needed, the rats used in these studies were approximately 80 g in weight. Approximately 2 g of the liver was homogenized with 8 ml of cold 0.1 M potassium phosphate buffer, pH 7.8, containing 0.001 M disodium EDTA. The homogenate was centrifuged at 15,000*g* for 10 min. The resulting supernatant fraction was then centrifuged at 105,000*g* for 75 min. The supernatant fraction was decanted and the tubes were allowed to drain for 1 or 2 min. The microsomal pellet was homogenized with 2.0 ml of 0.03 M potassium phosphate buffer, pH 7.8, containing 0.001 M disodium EDTA. The microsomal suspension (0.1 ml) was pipetted into a glass scintillation vial and dissolved in 1 ml of NCS solubilizer (Amersham-Searle Corp.). Then 10 ml of toluene scintillation fluid containing 0.02% *p*-bis-[2-(4-methyl-5-phenyloxazoyl)]-benzene and 0.4% 2,5-diphenyloxazole was added and radioactivity was measured in a Nuclear-Chicago Model Mark I scintillation spectrometer. The counting efficiency for tritium was 49%.

In the determination of the biological half-life of phenobarbital, the rats were killed at various intervals after administering 5-ethyl-5-phenylbarbituric-2-^{14}C acid (New England Nuclear Corp.) and the drug was extracted into chloroform from plasma and liver homogenate as described by Goldbaum (8). An aliquot of chloroform was pipetted into a glass scintillation vial, dried, and was counted after adding 10 ml of toluene scintillation fluid. The half-life of caffeine-1-methyl-^{14}C (New England Nuclear Corp.) was similarly determined after extracting caffeine into benzene by the method of Axelrod and Reichenthal (9). The benzene was not removed from the vial before counting since it does not quench. The counting efficiency for ^{14}C was 85%.

In the *in vivo* experiments, the duration of action of zoxazolamine or methyprylon was recorded as the duration of loss of righting reflexes of the rat. The urinary 17-hydroxycorticosteroids and 6β-hydroxycortisol levels were measured by the method of Kuntzman *et al.* (10).

RESULTS

Evidence for or against de novo *synthesis of microsomal drug-metabolizing enzyme system by caffeine.* In a previous communication (3) we reported that the stimulatory effect of caffeine on the drug-metabolizing activity by the rat liver was counteracted by the simultaneous administration of ethionine or puromycin. Data presented in Table I show that actinomycin D also prevented caffeine from stimulating the *in vitro* metabolism of acetanilide and *o*-nitroanisole by the liver enzyme system.

Not only does caffeine fail to stimulate the drug-metabolizing activity when added

TABLE I

EFFECT OF ACTINOMYCIN D ON MICROSOMAL ENZYME INDUCTION[a]

Treatment	Product formed (μmoles/g protein ± SD)	
	Acetanilide	*o*-Nitroanisole
Vehicle (water) only	7.7 ± 1.0	7.4 ± 2.9
Actinomycin D	8.8 ± 2.6	4.4 ± 2.2
Caffeine	20.7 ± 10.0[b]	12.9 ± 2.3[b]
Caffeine + actinomycin D	10.5 ± 3.9	7.3 ± 3.2

[a] Rats (200 g) were treated orally four times with 75 mg/kg of caffeine (4 PM first day, 9 AM and 4 PM second day, and 9 AM third day) and were killed 4 hr after the last dose. Rats receiving actinomycin D (10 μg per injection, ip) were injected four times according to a schedule identical to that of caffeine treatment. Four rats were used per group. Unless otherwise indicated, all experiments in the present studies were carried out with postmitochondrial supernatant fraction.

[b] Significantly different from vehicle control, $p < .05$.

directly to an incubation mixture, but there is a time lag after its administration to the rat before the stimulatory effect on drug metabolism becomes apparent. As shown in Table II, an increase in the rate of drug metabolism was not evident 4 hr after a single dose of caffeine but was demonstrable after 7 hr for acetanilide hydroxylase activity. When *o*-nitroanisole was used as a substrate, caffeine had an initial inhibitory effect 4 hr after a single dose and then a stimulatory effect after two doses.

TABLE II

TIME COURSE OF INDUCTION AFTER CAFFEINE ADMINISTRATION[a]

No. doses of caffeine	No. of days on caffeine	Product formed (μmoles/g protein ± SD)	
		o-Nitroanisole	Acetanilide
0	—	6.1 ± 0.3	11.2 ± 1.9
1	<½	4.5 ± 0.8[b]	13.5 ± 2.1
1	<½[c]	6.5 ± 0.9	16.9 ± 1.5[b]
2	1	7.7 ± 1.1[b]	18.8 ± 1.5[b]
4	2	8.8 ± 1.5[b]	22.9 ± 2.7[b,d]
6	3	11.6 ± 3.8[b]	25.7 ± 2.5[b,d]

[a] Four rats (300 g) in each group were given caffeine orally (38 mg/kg per dose). Except as noted, all experimental rats were killed 4 hr after the last caffeine dose. Control rats were given water twice daily for 3 days.

[b] Significantly different from the control, $p < .05$.

[c] Killed 7 hr after the last dose of caffeine.

[d] Not significantly different from each other.

A comparative study on the effects of caffeine and phenobarbital on the rat liver (Table III) shows that although the hydroxylase and *N*-demethylase activities were similarly stimulated by the two compounds, increases in the liver weight and microsomal protein or CO-binding pigment content in the liver were observed only with phenobarbital.

Examination of the effects of the two compounds on the *in vivo* incorporation of labeled amino acid into the microsomal proteins showed that, unlike phenobarbital, caffeine did not appreciably increase the rate of protein synthesis (Table IV). Although the data are not presented, caffeine did not stimulate the incorporation of labeled uridine into the liver nuclear ribonucleic acid, but phenobarbital did have a stimulatory effect under the same conditions.

Interaction of caffeine with the liver microsomes. Although evidence was not obtained to show that caffeine stimulated the synthesis of a constituent of the microsome, it could be shown that caffeine acted primarily, if not exclusively, on the microsomal frac-

TABLE III

EFFECT OF PHENOBARBITAL AND CAFFEINE ON RAT LIVER MICROSOMES

Treatment[a]	Final body weight (g)	Liver weight (g/100 g)	Microsomal protein (mg/g of liver)	CO-binding pigment[b] (mμmole/mg of protein)	Acetanilide hydroxylase (μmoles/g protein)	*p*-Chloro-*N*-methylaniline *N*-demethylase (μmoles/g protein)
Saline (0.2 ml), ip, twice daily	222 ± 15	4.64 ± 0.25	16.89 ± 0.86	0.341 ± 0.114	11.9 ± 1.9	44.9 ± 2.5
Phenobarbital (38 mg/kg), ip, twice daily	246 ± 17 $p < .05$	5.52 ± 0.36 $p < .001$	21.74 ± 2.37 $p < .001$	0.912 ± 0.113 $p < .001$	27.7 ± 2.7 $p < .001$	61.4 ± 3.6 $p < .001$
Saline (0.5 ml), oral, twice daily	221 ± 5	4.52 ± 0.18	17.48 ± 2.24	0.427 ± 0.145	9.0 ± 2.1	33.5 ± 3.[illegible]
Caffeine (75 mg/kg), oral, twice daily	225 ± 24	4.56 ± 0.17	17.25 ± 2.52	0.570 ± 0.270	24.5 ± 5.3 $p < .001$	46.4 ± 6.2 $p < .005$

[a] Six rats (200 g) in each group were treated as indicated for 4½ days. The interval between the last dose and sacrifice was 4 hr for caffeine-treated rats and 5 hr for phenobarbital-treated rats. The results are expressed as the mean ± SD.

[b] Based on molar extinction of 91 cm^{-1} mM^{-1} at 450 mμ (11).

TABLE IV

EFFECT OF CAFFEINE AND PHENOBARBITAL ON *o*-NITROANISOLE *O*-DEMETHYLATION AND ON THE INCORPORATION *in Vivo* OF PHENYLALANINE INTO THE MICROSOMES[a]

Days on drug: No. of injections:	1 / 2		1½ / 3		2 / 4	
Caffeine	90,200 ± 12,300* [7.6 ± 1.2]***	(4)	75,200 ± 7,600 [7.6 ± 0.4]***	(5)	78,300 ± 7,700 [7.6 ± 2.3]**	(5)
Phenobarbital	131,800 ± 30,100*** [9.7 ± 2.1]***	(5)	100,300 ± 15,000** [11.4 ± 2.9]***	(5)	107,600 ± 15,000*** [10.6 ± 1.6]***	(5)
Control			78,800 ± 6,200 [4.9 ± 0.6]	(6)		

* $p < .05$.
** $p < .01$.
*** $p < .005$.

[a] Rats (80 g) were injected ip with either saline, caffeine (75 mg/kg), or phenobarbital (38 mg/kg) for the number of days indicated. Five hours after the last medication, the rats were injected ip with 10 μCi of L-phenylalanine-2,3-^{3}H (1000 mCi/mM) and were killed 90 min after L-phenylalanine injection. The results are expressed as cpm/liver ± SD. Figures in brackets are μmoles/g protein ± SD of *o*-nitrophenol formed. Figures in parentheses refer to the number of rats per group.

TABLE V

EFFECT OF CAFFEINE AND PHENOBARBITAL ON THE INTRACELLULAR FRACTIONS[a]

Source of enzyme fraction: Supernatant	Source of enzyme fraction: Microsome	*p*-Hydroxyacetanilide formed (μmoles/g protein)	Relative activity
Control	Control	7.58	100
Caffeine	Control	7.95	104
Control	Caffeine	12.39	163
Caffeine	Caffeine	16.08	212
Control	Control	5.64	100
Phenobarbital	Control	7.84	140
Control	Phenobarbital	15.10	268
Phenobarbital	Phenobarbital	17.29	307

[a] Rats (200 g) were given orally 75 mg/kg of caffeine twice daily for 3½ days (seven times) or were injected ip with 38 mg/kg of phenobarbital for 4½ days (nine injections). The rats were killed 4 hr after the last medication. The corresponding control rats were treated with water. The microsomal fraction, which was washed once with isotonic KCl, and the 100,000 *g* supernatant fraction were prepared and were incubated in various combinations. The results shown are the averages from two experiments.

TABLE VI

EFFECT ON THE HEPATIC DRUG-METABOLIZING ACTIVITIES OF CAFFEINE AND PHENOBARBITAL ADMINISTERED SEPARATELY AND SIMULTANEOUSLY[a]

Group	Product formed (μmoles/g protein ± SD): Acetanilide	*o*-Nitroanisole	Aminopyrine
Saline	5.4 ± 2.1	11.4 ± 1.4	5.0 ± 2.0
Caffeine	15.4 ± 1.0	16.1 ± 3.1	9.9 ± 1.4
	$p < .001$	$p < .05$	$p < .01$
Phenobarbital	14.8 ± 2.2	20.9 ± 2.1	25.6 ± 3.6
	$p < .001$	$p < .001$	$p < .001$
Caffeine + phenobarbital	23.1 ± 1.5[b]	22.9 ± 2.4	26.7 ± 1.2
	$p < .001$	$p < .001$	$p < .001$

[a] Four rats (170 g) were used per group. The rats were injected ip with saline, caffeine (75 mg/kg, twice daily), phenobarbital (38 mg/kg, twice daily) or with a mixture of caffeine and phenobarbital (twice daily) for 3 days.

[b] Significantly different from caffeine or phenobarbital values at $p < .001$.

tion. Thus, fractionation of livers from control and caffeine-treated rats into the microsomal fraction and 100,000*g* supernatant fraction and cross-over recombination experiments indicated that the stimulatory effect of caffeine on acetanilide hydroxylase was exhibited primarily by the microsomal fraction. A similar finding was obtained with phenobarbital (Table V).

Since both caffeine and phenobarbital exert their effects primarily on the microsomal fraction, it was of interest to examine whether the effect of caffeine is

additive to that of phenobarbital. As shown in Table VI, the stimulatory effect of the mixture of the two compounds exceeded that of caffeine or phenobarbital only when acetanilide was the substrate.

Although there does not seem to be a direct relationship between how good an inducer a compound is and the rate at which it is metabolized by the microsomal enzymes, most inducers serve as substrates of the microsome. Caffeine was found to be slowly converted to highly polar metabolite(s) by the microsomal system and this reaction was stimulated by pretreating the rat with phenobarbital (Table VII). Caffeine pretreatment had no effect on the metabolism of caffeine under these conditions.

TABLE VII

In Vitro METABOLISM OF CAFFEINE[a]

Groups	Caffeine metabolized (μmoles/g protein ± SD)
Control	0.52 ± 0.14
Phenobarbital	1.93 ± 0.27
	$p < .01$

[a] Portions of livers from the same rats used in experiments described under Table V were used. Caffeine-1-methyl-^{14}C (0.05 μCi in 10 μmoles) was incubated with postmitochondrial supernatant fraction. The incubation mixture (3 ml) was saturated with NaCl and extracted with 5 ml of benzene to remove unreacted caffeine. The mixture was extracted again with 3 ml of benzene after adding a few milligrams of solid caffeine and once with 5 ml of washed *n*-butanol. An aliquot of the residual aqueous layer was counted in a liquid scintillation counter using Bio-Solv (BBS-3 Beckman) and toluene scintillation fluid. The counting efficiency for ^{14}C was 80%. There were four animals in each group.

Duration of the elevated microsomal activity after caffeine treatment. One of the peculiarities of the action of caffeine is its short duration of effectiveness as a stimulatory agent after the termination of treatment. We found that the stimulatory effect of phenobarbital on drug-metabolizing activities in the rat lasted for 5–7 days after the termination of 3-day pretreatment. In contrast to this, the effect of caffeine on the enzymic activity lasted for only 24 hr after the last dose (Table VIII).

The shorter duration of action of caffeine in comparison with phenobarbital may be explained by its shorter biological half-life in the rat. Data presented in Figs. 1 and 2 indicate that after 3 days of pretreatment of the rat, the biological half-life of phenobarbital was approximately four times that of caffeine.

Effect of caffeine on the in vivo *response to drugs.* The duration of sleep induced by hexobarbital administration was not shortened by pretreating the rat with caffeine. In agreement with this observation, the hepatic enzyme system of the caffeine-treated rat did not metabolize hexobarbital at a higher rate than that of the untreated rat. However, the duration of action of zoxazolamine and methyprylon was significantly less in the caffeine-treated rat than in the control rat (Table IX). The onset of the action of the drugs was not affected by caffeine pretreatment.

Effect of intake of coffee or tea on the excretion of 6β-hydroxycortisol by man. To

TABLE VIII

DURATION OF ELEVATED MICROSOMAL ACTIVITY AFTER CAFFEINE AND PHENOBARBITAL ADMINISTRATION[a]

Compound administered	Days after medication	Product formed (μmoles of product/g protein ± SD)		
		Acetanilide	*o*-Nitroanisole	Aminopyrine
None (10)	—	8.6 ± 2.3	9.6 ± 2.0	2.2 ± 0.9
Caffeine (5)	1	14.7 ± 2.8[b]	12.8 ± 3.4[b]	4.7 ± 1.5[b]
(5)	3	7.6 ± 1.3	8.5 ± 1.4	2.3 ± 1.1
Phenobarbital (3)	1	18.6 ± 2.3[b]	21.0 ± 1.5[b]	17.7 ± 1.0[b]
(5)	3	15.5 ± 1.4[b]	16.8 ± 1.4[b]	10.2 ± 2.4[b]

[a] Rats (185–210 g) were administered either caffeine orally (75 mg/kg) or phenobarbital ip (30 mg/kg) twice daily for 3 days. The rats were sacrificed 1 day or 3 days after the last medication. Figures in parentheses refer to the number of rats in each group.

[b] Significantly different from the control values at $p < .05$.

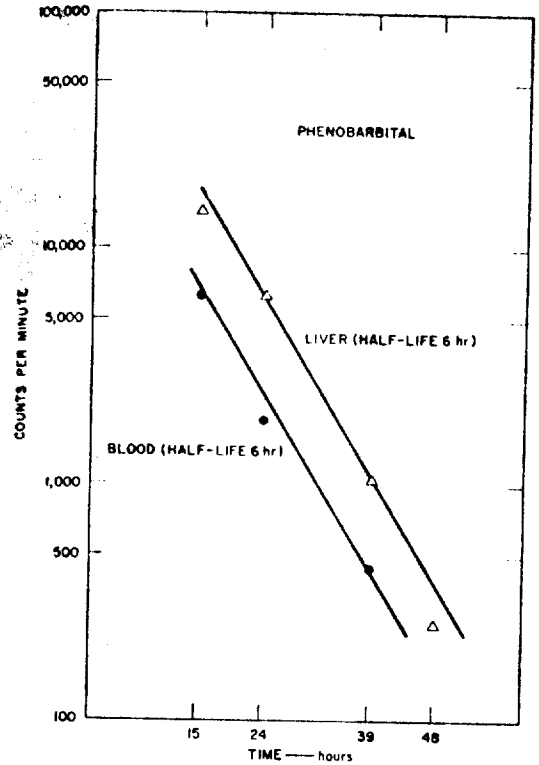

FIG. 1. Half-life of phenobarbital in the rat. Rats (300 g) were injected ip with 38 mg/kg of phenobarbital twice daily for 3 days. With the last (sixth) dose 10 μCi of 5-ethyl-5-phenylbarbituric-2-^{14}C acid were administered. Each point represents an average of closely agreeing duplicates.

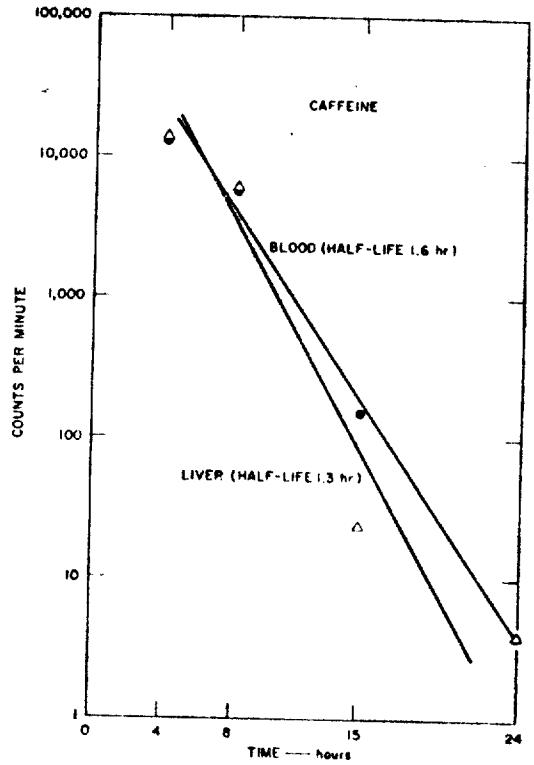

FIG. 2. Half-life of caffeine in the rat. The experimental procedures were the same as described under Fig. 1 except that 50 mg/kg of caffeine and caffeine-1-methyl-^{14}C were used.

obtain indication as to whether caffeine acts as a microsomal enzyme inducer in man, nonsmoking volunteers were recruited and the amounts of 6β-hydroxycortisol excretion by these individuals in the urine before and after coffee or tea were measured. The subjects were requested to abstain from drinking caffeine-containing beverages for 5 to 6 days before collecting their first 24-hr specimens. They were then asked to drink a minimum of six cups of coffee or tea daily for 3-4 days; during the last day of this period the second 24-hr specimens were collected. As can be seen from the data presented in Table X, the intake of caffeine-containing beverages may have caused some increase in the amount of 6β-hydroxycortisol excreted. However, the increment is not large enough to accept it as a positive indication of enzyme induction.

TABLE IX

EFFECT OF CAFFEINE ON DURATION OF ACTION OF DRUGS

Drug	Group	Duration of drug action (min ± SD)
Hexobarbital[a] (100 mg/kg)	Control (10)	30 ± 5
	Experimental (10)	32 ± 6
		NS
Zoxazolamine[a] (60 mg/kg)	Control (6)	142 ± 29
	Experimental (6)	97 ± 18
		$p < .01$
Methyprylon[b] (135 mg/kg)	Control (7)	282 ± 52
	Experimental (8)	181 ± 52
		$p < .005$

[a] Rats were allowed to drink water containing caffeine (1 mg/ml) for 3 days. The average daily intake was 125 mg/kg caffeine per rat. The weights of the rats were 210 and 250 g, respectively, for the hexobarbital and zoxazolamine experiments.

[b] Rats were injected ip with 75 mg/kg caffeine twice daily for 3 days. The weights of the rats were 160 g.

TABLE X

EFFECT OF CAFFEINE ON 17-HYDROXYCORTICOSTEROIDS (17-OHCS) AND 6β-HYDROXYCORTISOL (6-OH CORTISOL) EXCRETION IN HUMAN SUBJECTS

	Before caffeine intake			During caffeine intake		
	Creatinine (g/day)	17-OHCS (mg/day)	6-OH cortisol (μg/day)	Creatinine (g/day)	17-OHCS (mg/day)	6-OH cortisol (μg/day)
Male subjects						
A	2.00	4.21	360	1.92	4.25	415
B	1.64	6.23	340	1.73	6.33	390
C	2.09	8.79	260	1.86	11.03	240
D	1.17	4.77	150	1.45	3.80	210
Female subjects						
E	1.24	2.65	150	1.29	2.42	200
F	1.32	6.65	135	1.46	7.27	210
G	1.23	4.39	135	1.09	2.67	190
H	1.24	3.59	215	1.46	4.35	290

DISCUSSION

The data presented in this paper demonstrate that caffeine stimulates drug metabolism in rats. This was shown by the increased rate of drug metabolism by the hepatic microsomal system of the rat pretreated with caffeine. Caffeine pretreatment also shortened the duration of action of zoxazolamine and methyprylon, both of which are known to be metabolized by the hepatic enzyme system (12, 13). The duration of sleeping time after hexobarbital administration, however, was not affected by caffeine. In this respect, caffeine was similar to 3,4-benzpyrene, which also stimulated the metabolism of zoxazolamine but not that of hexobarbital (14).

Indirect evidence indicated that caffeine acted by stimulating the *de novo* synthesis of the microsomal enzymes. The stimulatory effect of caffeine on the microsomal enzyme was nullified by the simultaneous administration of inhibitors of protein synthesis. Furthermore, a definite time lag after caffeine administration was observed before the stimulatory effect on drug metabolism could be demonstrated. Adding caffeine directly to an incubation mixture or fortifying the mixture with cyclic 3′, 5′-AMP or its dibutyryl derivative was ineffective. The cyclic 3′, 5′-AMP has been shown to stimulate steroid 11β-hydroxylase activity in rat adrenal homogenates (15). No evidence was obtained for the involvement of cyclic AMP in drug metabolism.

Attempts to obtain more direct evidence for caffeine as an inducer of the microsomal enzymes have not been successful. We were not able to show that caffeine, like phenobarbital, stimulated the incorporation of labeled amino acid into the microsomes or increased the cytochrome P-450 content of the liver.[2] These results cannot readily be reconciled with those obtained with inhibitors of protein synthesis. It is of interest that theophylline, which we have shown to be somewhat less effective than caffeine as an inducer (3), was reported to stimulate amino acid incorporation into protein in rat liver in an *in vitro* system (15). The kinetic data previously reported, using acetanilide as the substrate, did not support the hypothesis that caffeine facilitated the penetration of a substrate through the microsomal membrane since the K_m value for acetanilide was identical with or without caffeine treatment.

Another possible approach in investigating whether caffeine and phenobarbital evoke their stimulatory effects through similar or different mechanisms is to examine the effects of administering these two compounds together. The results obtained were equivocal since the effects of the two

[2] Recent studies indicated that after caffeine pretreatment, there was an increase in the 455-mμ peak of the difference spectra of reduced microsomal homoprotein complexed with ethyl isocyanide but not in the 430-mμ peak. These data will be reported in a future communication.

agents appeared to be additive only in the case of acetanilide but not when *o*-nitroanisole and aminopyrine were the substrates.

Until further studies prove otherwise, caffeine appears to be stimulating the microsomal enzymes without causing a net increased synthesis of the enzymes. An example of a compound that causes synthesis of an enzyme with a higher specific activity without an increase in the enzyme level has been described in the literature (17).

An initial attempt to obtain evidence for induction of the microsomal enzyme in man by looking for an increased excretion of 6β-hydroxycortisol in the urine gave negative results. Whether a larger daily intake or a more prolonged intake of caffeine would have an effect on the enzyme system in man must await further experiments.

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REFERENCES

1. Conney, A. H., *Pharmacol. Rev.* **19**, 317 (1967).
2. Rubin, E., Hutterer, F., and Lieber, C. S., *Science* **159**, 1469 (1968).
3. Mitoma, C., Sorich, T. J., II, and Neubauer, S. E., *Life Sci.* **7**, 145 (1968).
4. Mitoma, C., Neubauer, S. E., Badger, N. L., and Sorich, T. J., II, *Proc. Soc. Exptl. Biol Med.* **125**, 284 (1967).
5. Tagg, J., and Mitoma, C., *Biochem. Pharmacol.* **17**, 2471 (1968).
6. Kupfer, D., and Bruggeman, L. L., *Anal. Biochem.* **17**, 502 (1966).
7. Schenkman, J. B., Frey, I., Remmer, H., and Estabrook, R. W., *Mol. Pharmacol.* **3**, 516 (1967).
8. Goldbaum, L. R., *Anal. Chem.* **24**, 1604 (1952).
9. Axelrod, J., and Reichenthal, J., *J. Pharmacol. Exptl. Therap.* **107**, 519 (1953).
10. Kuntzman, R., Jacobson, M., Levin, W., and Conney, A. H., *Biochem. Pharmacol.* **17**, 565 (1968).
11. Omura, T., and Sato, R., *J. Biol. Chem.* **239**, 2379 (1964).
12. Conney, A. H., Trousof, N., and Burns, J. J., *J. Pharmacol. Exptl. Therap.* **128**, 333 (1960).
13. Randall, L. O., Iliev, V., and Brandman, O., *Arch. Intern. Pharmacodyn.* **106**, 388 (1956).
14. Conney, A. H., Davison, C., Gastel, R., and Burns, J. J., *J. Pharmacol. Exptl. Therap.* **130**, 1 (1960)
15. Creange, J. E., and Roberts, S., *Biochem. Biophys. Res. Commun.* **19**, 73 (1965).
16. Raghupathy, E., and Searle, G. L., *Federation Proc.* **28**, 473 (1969).
17. Griffin, M. J., and Cox, R. P., *Proc. Natl. Acad. Sci. U.S.* **56**, 946 (1966).

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EFFECT OF ENVIRONMENTAL TEMPERATURE ON THE TOXICITY OF CAFFEINE AND DEXTROAMPHETAMINE IN MICE

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ABSTRACT

MULLER, PATRICIA J. AND JOAN VERNIKOS-DANELLIS: Effect of environmental temperature on the toxicity of caffeine and dextroamphetamine in mice. J. Pharmacol. Exp. Ther. **171**: 153–158, 1970. The effects of environmental temperature and dehydration, individually and in combination, on the toxicity of caffeine and dextroamphetamine were studied in mice. LD50's were compared at 22°C (normal), 30°C (warm) and 15°C (cool), with and without dehydration. No statistically significant difference in LD50's ($P < .05$) could be shown between acute (one hour) and subacute (three days) preinjection exposures. A 7–8° alteration in temperature on either side of 22°C significantly changed the toxicity of both drugs. At 15°C the toxicity of dextroamphetamine decreased 10-fold, and the animals were protected from its toxic effects; at 30°C toxicity increased 2- to 3-fold. Both warming and cooling appeared to increase the toxicity of caffeine. Dehydration caused a marked decrease in the LD50's of both drugs. This decrease was more pronounced at the higher temperatures, with the exception of dextroamphetamine at 15°C where cooling appeared to mask the increase in toxicity otherwise produced by dehydration. The combination of dehydration and change in temperature showed an additive effect on the toxicity of caffeine at 15°C and dextroamphetamine at 30°C, whereas a marked potentiation occurred with caffeine at 30°C. These results indicate that alterations of the environmental temperature markedly affect drug toxicity. They emphasize that such alterations do not have to be particularly drastic but that "mild" variations in temperature are effective.

Numerous reports in the literature have demonstrated that genetic and environmental factors alter the responsiveness of animals to drug toxicity (Setnikar and Temelcou, 1962), duration (Rupe *et al.*, 1963) and metabolism (Stitzel and Furner, 1967). Types of stresses used have included hind leg ligation (Rupe *et al.*, 1963), shock (Moore and Lariviere, 1964), aggregation (Clark *et al.*, 1967), starvation (Peters, 1966), high altitude (Baumel *et al.*, 1967) and variation in environmental temperature (Keplinger *et al.*, 1959; Chen *et al.*, 1943; Hohn and Lasagna, 1960). Changes in temperature have generally been drastic (4–37°C), and the effects of temperature on drug action have been extensively reviewed (Fuhrman and Fuhrman, 1961). Current literature (Barry and Buckley, 1966) suggests the usefulness of determining the effect of stressors on drug toxicity as an aid in determining appropriate doses during stress and normal conditions.

The present investigation was conducted to study the effects of mild environmental temperature changes and dehydration, as well as combinations of these, on the toxicity of two central nervous system stimulants, caffeine and dextroamphetamine.

METHODS. Male Swiss-Webster mice, weighing 25 to 30 g, were used. They were housed in a Lab-Line Environmental Room at constant temperature, 50% relative humidity, and 12-hour light:12-hour dark cycles, and were given food and water *ad libitum* until the time of injection. Because the variability in the toxicity of dextroamphetamine has been reported to depend on the number of mice per cage (Hohn and Lasagna, 1960), throughout this study the animals were housed 10 per cage in opaque plastic cages having a floor size of 30 by 36 cm. The dose of drug was dissolved in 0.45 ml of distilled water per 30 g of mouse weight. Twenty mice were used per dose level. Uninjected and physiologic saline (0.45 ml per 30 g body weight) injected controls were run simultaneously.

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The [illegible] were injected i.p. at 10:00 A.M., four hours after lights-on, to minimize the circadian variation in drug toxicity (Halberg, 1960; Reinberg, 1967). Twenty-four-hour LD50's were selected because, beyond this time, no further deaths occurred, except with dehydration where animals were followed until 100% mortality occurred.

Normal environmental temperature was considered to be 22°C. Drug toxicity was studied in animals exposed to warm (30°C) and cool (15°C) temperatures. Acute (one hour) and subacute (three days) preinjection exposures were used. The exposures continued postinjection until the termination of the study. Dehydration consisted of a three-day preinjection withdrawal of water only and continued postinjection for five days in the study of survival rates. Body temperatures of a similarly exposed group of mice were taken rectally at 2, 4, 6, 8 and 24 hours, and at 2 and 3 days, using a Yellow Springs no. 402 Thermister Probe and a no. 43 Telethermometer readout box. This was done to determine whether any change in drug toxicity was a result of hyper- or hypothermia of the animal due to the environment (Welch, 1968). Rectal temperature elevation at 30°C and depression at 15°C differed less than a degree from the 22°C controls. The temperature returned and stabilized at pre-exposure levels after 24 hours.

LD50's were derived by probit analysis (Finney, 1952) from five points on a log-dose scale with 20 mice per point.

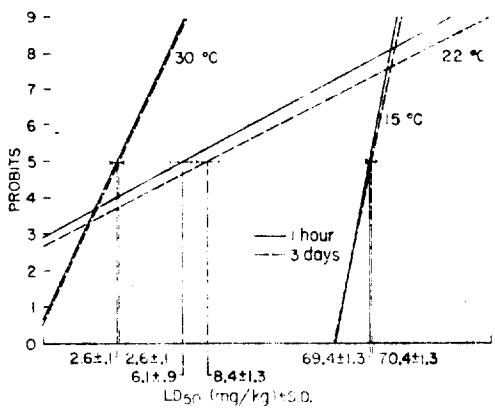

FIG. 1. LD50's of dextroamphetamine at three temperatures, using a one-hour and three-day preinjection exposure period.

RESULTS. Figure 1 shows a comparison of log-dose probit plots and LD50's for dextroamphetamine at three temperatures and two exposure periods. Figure 2 shows a similar comparison for caffeine. No statistically significant difference could be shown between the one-hour and three-day exposure times ($P > .05$). At 15°C dextroamphetamine toxicity was approximately 10 times less than at 22°C, whereas at 30°C there was a 2- to 3-fold increase in toxicity. Both warming and cooling appeared to increase the toxicity of caffeine (fig. 2). The effect was significant ($P < .05$) at 30°C but only at the 90% level for cooling.

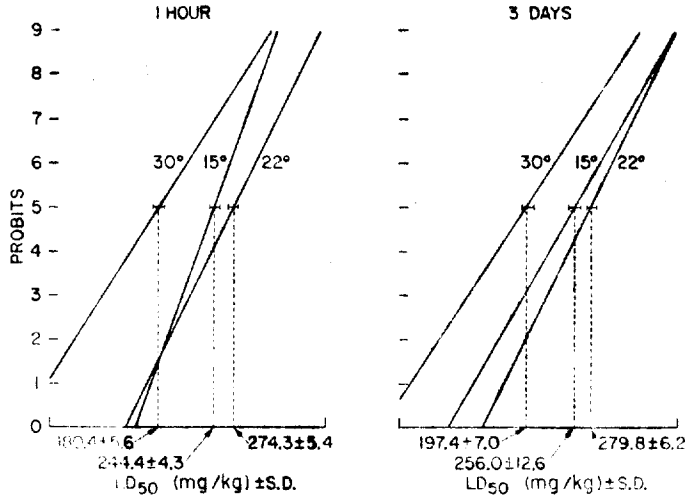

FIG. 2. LD50's of caffeine at three temperatures after one-hour and three-day preinjection exposure periods.

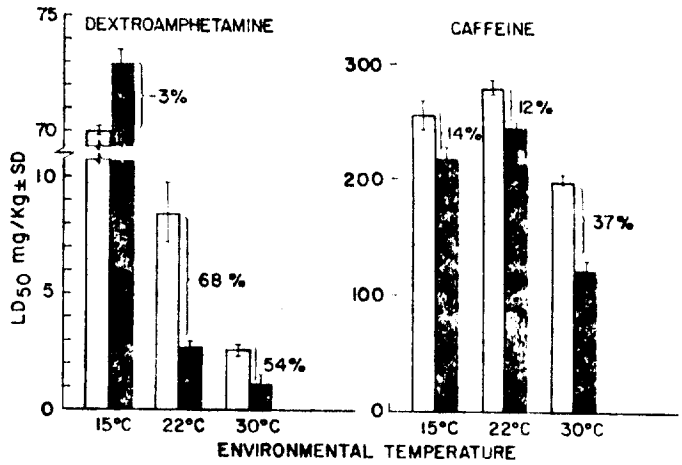

FIG. 3. LD50's in nondehydrated (white) and dehydrated (gray) mice at temperatures studied. Percentage represents change in LD50 from nondehydrated value.

Figure 3 compares the LD50 of both caffeine and dextroamphetamine in nondehydrated and dehydrated mice at the three temperature levels. Whereas the toxicity of caffeine was least affected by dehydration at normal (22°C) temperature, dextroamphetamine-treated animals showed the greatest susceptibility to dehydration at this temperature. In fact, at 15°C, cooling appeared to prevent the increase in toxicity of dextroamphetamine.

The effect of potentiation and the addition of stresses on drug toxicity are illustrated in figure 4. The toxicity of caffeine in mice exposed to cold and dehydration or of dextroamphetamine in mice exposed to heat and dehydration appears to be merely additive. On the other hand, exposure to heat and dehydration increased the toxicity of caffeine far in excess of the predicted value, derived from the sum of dehydration and heat alone, clearly showing the potentiating effect of the two adverse environments. As mentioned above, the effect of cold completely masked the effect of dehydration on the toxicity of dextroamphetamine.

The effect of a compound stress on drug toxicity indicates how unpredictable the outcome of an interaction can be. The lack of similarity in the action is clearly illustrated when the survival curves in figure 5 are examined. Prolonging the dehydration for several days postinjection alters the survival rate of the drug-treated mice at the temperature studied. The caffeine-treated mice continue to die at a rapid

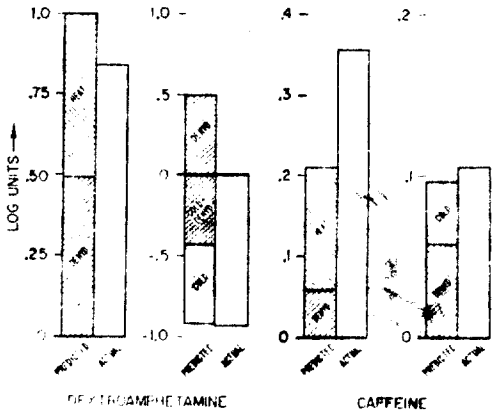

FIG. 4. Combined stress interaction. Comparison of the actual value (log LD50 of controls minus log LD50 of combined stresses) and predicted value (sum of the differences obtained by subtracting each individual stress log LD50 from the control log LD50).

rate, whereas the survival rate of the dextroamphetamine-treated mice tended to plateau for up to five days, except at the cool temperature at which the toxicity may possibly have involved different mechanisms.

DISCUSSION. Most of the studies determining the effects of environmental temperature on drug toxicity have involved exposure of animals to drastic conditions that had a profound effect on the body temperature of the animal (Ingenito and Bonnycastle, 1967). Relatively few studies have involved moderate temperature changes in

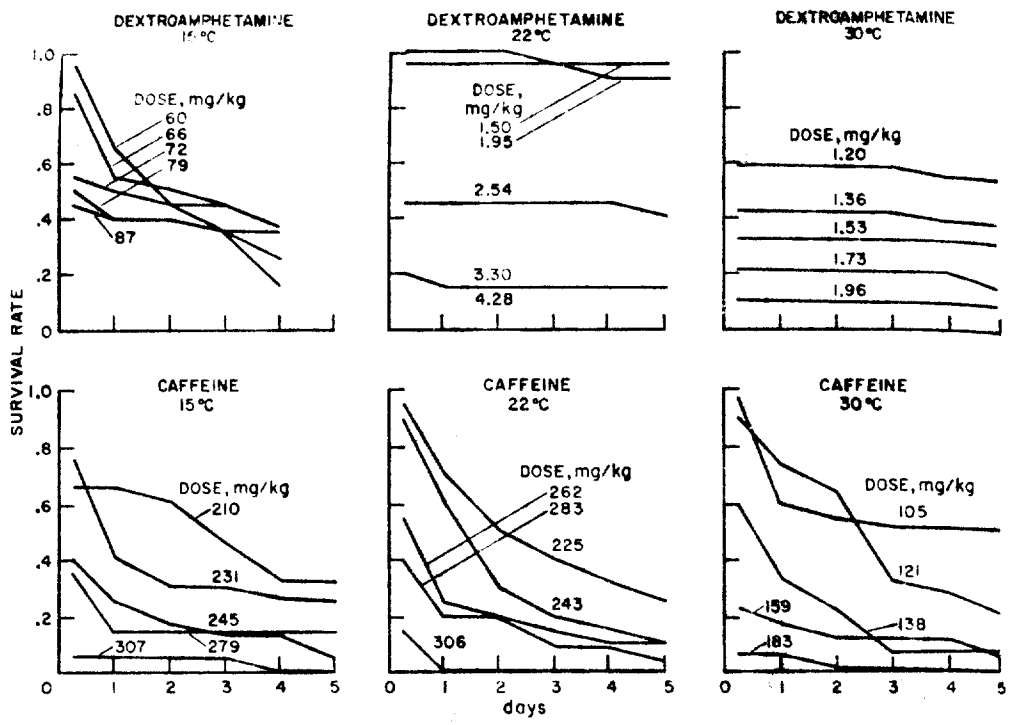

FIG. 5. Survival rates of drug-injected dehydrated mice (corrected for control losses).

the environment (Stahnke, 1965; Chance, 1947; Chen *et al.*, 1943). Although an initial change in body temperature occurred in the present study, it did not appear to affect the drug toxicity since similar results were obtained with one hour and three days of exposure.

In this study, minor variations in temperature were used as a mild stress to the organism in order to see whether the toxicity followed a general pattern of stress-drug interaction or a specific action characteristic of the stress applied. The toxicity of caffeine in mice at 22°C agrees quantitatively with that found in the rat (Keplinger *et al.*, 1959), although Keplinger *et al.* used greater temperature changes in their study for hot and cold exposures. Contrary to Keplinger and his co-workers' finding that cooling caused no change in toxicity, a slight increase was noted in the toxicity of caffeine at a cool temperature, suggesting that both heat and cold increase the toxicity of caffeine. This result supports the view that the toxicity of caffeine followed a nonspecific pattern of stress-drug interaction, although this was not borne out by the results with dextroamphetamine. The increased toxicity of dextroamphetamine caused by an increase in environmental temperature agrees with observations made by other investigators (Chance, 1947; Hohn and Lasagna, 1960). The data clearly show, as in the work of Keplinger *et al.*, that the effect of temperature on drug toxicity depends upon the drug involved and the temperature used. However, our work shows that extreme temperatures are not necessary since profound effects can be achieved by mild temperature variation.

Although the mechanism of dextroamphetamine action in the central nervous system is not definitely established, considerable evidence indicates that it is mediated by catecholamines. The persistence of central actions of amphetamine in reserpine-treated animals has been interpreted to indicate direct receptor-activating properties (Vane, 1960; van Rossum *et al.*, 1962; Smith, 1963). However, the synthesis of dopamine (and possibly norepinephrine) in brain appears to persist in the reserpine-treated animal (Andén *et al.*, 1964), and thus indirect actions cannot be ex-

cluded. Furthermore, investigators (Carlsson, 1965; Carlsson *et al.*, 1966) have shown that low doses of amphetamine are capable of releasing catecholamines in brain, even in reserpine-treated animals in which catecholamine levels have been increased by treatment with L-dopa. Inhibition of brain norepinephrine synthesis by the tyrosine hydroxylase inhibitor, α-methyltyrosine, has been shown to reduce the central stimulant effects of dextroamphetamine (Menon *et al.*, 1967) and to reduce its toxicity in aggregated mice (Mennear and Rudzik, 1968). Similar protection from the toxic effects of amphetamine could be obtained by such amine-depleting agents as reserpine, metaraminol and α-methyl-*meta*-tyrosine (Mennear and Rudzik, 1966). Exposing rats to various stressful agents, such as electric shock, ether anesthesia and cold (Vogt, 1954; Maynert and Levi, 1964), has been reported to result in large and rapid decreases in hypothalamic and brainstem norepinephrine content. The present reduced toxicity of dextroamphetamine in mice exposed to cold could possibly be explained on the basis of reduced brain norepinephrine. An alternative explanation on the effects of environmental temperature on drug toxicity could involve alterations in quantity of drug distributed, in the diffusion of the drug from the blood to the tissues and in the metabolism of the drug, as elegantly demonstrated by Setnikar and Temelcou (1962).

The exact mechanism for stress-drug interaction becomes difficult to explain since it involves looking at the end result of applying particular stressful situations without considering the intermediate events. The problem is compounded when changes in temperature are combined with a stress-like dehydration, where it might be anticipated that heat exchange and water balance would be altered. This becomes evident with a diuretic drug like caffeine, as shown by the survival rates in dehydrated heat-exposed animals.

The toxicity of amphetamine and other drugs has been shown to vary with circadian periodicity (Scheving *et al.*, 1968; Reinberg, 1967) and it has been pointed out repeatedly that this periodicity is an important factor that must be considered in the design of experiments to evaluate LD50's and drug toxicity in general. The principles of periodicity should also be applied in determining whether they affect the therapeutic use of the drug. Similarly, the present findings emphasize that it would be useful to take into consideration that the toxicity and effectiveness of a given drug may be potentiated, counteracted or unaffected by milder stresses than the common laboratory variety.

The mechanism by which stress affects drug toxicity has been attributed to a pathway involving the pituitary-adrenal system (Rupe *et al.*, 1963; Bousquet *et al.*, 1965; Driever and Bousquet, 1965). There is no doubt that both heat and cold stress will activate the pituitary-adrenal system (Kotby *et al.*, 1967; Knigge, 1960), as indicated by increased levels of plasma steroids. However, if the change in toxicity of a drug were indeed related to the intensity of the stress stimulus as mediated by the adrenal, then the direction and intensity of this change could be predicted, and both heat and cold would be expected to have the same effect. This is obviously not true, according to a comprehensive review of the effect of temperature on acute drug toxicity by Fuhrman and Fuhrman (1961). The present study also indicates that the alteration in drug toxicity is related instead, to the type of environmental change and the mechanism of action of the drug studied. Furthermore, when two or more stressful stimuli are combined, the effect on toxicity is not entirely predictable.

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REFERENCES

ANDÉN, N. E., ROOS, B. E. AND WERDINIUS, B.: Effects of chlorpromazine, haloperidol and reserpine on the levels of phenolic acids in rabbit corpus striatum. Life Sci. **3**: 149-158, 1964.

BARRY, H. AND BUCKLEY, J. P.: Drug effects on animal performance and the stress syndrome. J. Pharm. Sci. **55**: 1159–1183, 1966.

BAUMEL, I. P., ROBINSON, S. M. AND BLATT, W. F.: Multi-chamber system for toxicity studies in mice at simulated high altitude. J. Pharm. Sci. **56**: 918–919, 1967.

BOUSQUET, W. F., RUPE, B. D. AND MIYA, T. S.: Endocrine modifications of drug responses in the rat. J. Pharmacol. Exp. Ther. **147**: 376–379, 1965.

CARLSSON, A.: Drugs which block the storage of 5-hydroxytryptamine and related amines. *In* Handbook of Experimental Pharmacology, ed. by O. Eichler and A. Farah, pp. 529–592, Springler-Verlag, New York, 1965.

CARLSSON, A., FUXE, K., HAMBERGER, B. AND LINDQVIST, M.: Biochemical and histochemical studies of the effects of imipramine-like drugs and (+)-amphetamine on central and peripheral catecholamine neurons. Acta Physiol. Scand. **67**: 481–497, 1966.

CHANCE, M. R. A.: Factors influencing the toxicity of sympathomimetic amines to solitary mice. J. Pharmacol. Exp. Ther. **89**: 289–296, 1947.

CHEN, K. K., ANDERSON, R. C., STELDT, F. A. AND MILLS, C. A.: Environmental temperature and drug action in mice. J. Pharmacol. Exp. Ther. **79**: 127–132, 1943.

CLARK, W. C., BLACKMAN, H. J. AND PRESTON, J. E.: Certain factors in aggregated mice *d*-amphetamine toxicity. Arch. Int. Pharmacodyn. Thér. **170**: 350–363, 1967.

DRIEVER, C. W. AND BOUSQUET, W. F.: Stress-drug interactions: Evidence for rapid enzyme induction. Life Sci. **4**: 1449–1454, 1965.

FINNEY, D. J.: Probit Analysis, 2nd ed., Cambridge University Press, London, 1952.

FUHRMAN, G. J. AND FUHRMAN, F. A.: Effects of temperature on the action of drugs. Annu. Rev. Pharmacol. **1**: 65–78, 1961.

HALBERG, F.: Temporal coordination of physiologic function in biologic clocks. Cold Spring Harbor Symp. Quant. Biol. **25**: 289, 1960.

HOHN, R. AND LASAGNA, L.: Effects of aggregation and temperature on amphetamine toxicity in mice. Psychopharmacologia **1**: 210–220, 1960.

INGENITO, A. J. AND BONNYCASTLE, D. D.: The effect of exposure to heat and cold upon rat brain catecholamine and 5-hydroxytryptamine levels. Can. J. Physiol. Pharmacol. **45**: 733–743, 1967.

KEPLINGER, M. L., LANIER, G. E. AND DEICHMANN, W. B.: Effects of environmental temperature on the acute toxicity of a number of compounds in rats. Toxicol. Appl. Pharmacol. **1**: 156–161, 1959.

KNIGGE, K. M.: Neuroendocrine mechanisms influencing ACTH and TSH secretion and their role in cold acclimation. Fed. Proc. **19**: suppl. 5, 45–51, 1960.

KOTBY, S., JOHNSON, H. D. AND KIBLER, H. H.: Plasma corticosterone response to elevated environmental temperature (34°C) and related physiological activities as influenced by age. Life Sci. **6**: 709–719, 1967.

MAYNERT, E. W. AND LEVI, R.: Stress induced release of brain norepinephrine and its inhibition by drugs. J. Pharmacol. Exp. Ther. **143**: 90–96, 1964.

MENNEAR, J. H. AND RUDZIK, A. D.: The effects of amine depleting agents on the toxicity of amphetamine in aggregated mice. Life Sci. **5**: 349–356, 1966.

MENNEAR, J. H. AND RUDZIK, A. D.: The effect of alpha methyl meta tyrosine on the dose-lethality curve of *d*-amphetamine in aggregated mice. Life Sci. **7**: 213–218, 1968.

MENON, M. D., DANDUJA, P. C. AND BAFNA, J. S.: Modification of the effect of some central stimulants in mice pretreated with α-methyl-*l*-tyrosine. Psychopharmacologia **10**: 437–444, 1967.

MOORE, K. E. AND LARIVIERE, E. W.: Effects of stress and *d*-amphetamine on rat brain catecholamines. Biochem. Pharmacol. **13**: 1098–1100, 1964.

PETERS, J. M.: Caffeine toxicity in starved rats. Toxicol. Appl. Pharmacol. **9**: 390–397, 1966.

REINBERG, A.: The hours of changing responsiveness or susceptibility. Perspec. Biol. Med. **11**: 111–128, 1967.

ROSSUM, J. M. VAN, SCHOOT, J. B. VAN DER AND HURKMANS, J. A. TH.: Mechanism of action of cocaine and amphetamine in the brain. Experientia (Basel) **18**: 229–231, 1962.

RUPE, B. D., BOUSQUET, W. F. AND MIYA, T. S.: Stress modification of drug response. Science **141**: 1186–1187, 1963.

SCHEVING, L. E., VEDRAL, D. F. AND PAULY, J. E.: Daily circadian rhythm in rats to *d*-amphetamine sulfate: Effect of blinding and continuous illumination on the rhythm. Nature (London) **219**: 621–622, 1968.

SETNIKAR, I. AND TEMELCOU, O.: Effect of temperature on toxicity and distribution of pentobarbital and barbital in rats and dogs. J. Pharmacol. Exp. Ther. **135**: 213–222, 1962.

SMITH, C. B.: Enhancement by reserpine and α-methyl Dopa of the effects of *d*-amphetamine upon locomotor activity of mice. J. Pharmacol. Exp. Ther. **142**: 343–350, 1963.

STAHNKE, H. L.: Stress and the toxicity of venoms. Science **150**: 1456–1457, 1965.

STITZEL, R. E. AND FURNER, R. L.: Stress-induced alteration in microsomal drug metabolism in the rat. Biochem. Pharmacol. **16**: 1489–1494, 1967.

VANE J. R.: The actions of sympathomimetic amines in tryptamine receptors. *In* Adrenergic Mechanisms, ed. by J. R. Vane, G. E. W. Wolstenholme and M. O'Connor, pp. 356–372. J. & A. Churchill, Ltd., London, 1960.

VOGT, M.: The concentration of sympathin in different parts of the central nervous system under normal conditions and after the administration of drugs. J. Physiol. (London) **123**: 451–481, 1954.

WELCH, B. L.: Necessity of considering body temperature in drug-cold studies of catecholamines. Biochem. Pharmacol. **17**: 2013–2015, 19[illegible]

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COMMENTARY

Caffeine as Teratogen and Mutagen

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Caffeine is a likely mutagen and perhaps teratogen because of its chemical structure as a purine, one of the constituents of DNA. Caffeine can cross the human placenta and enter the fetal gonad (Goldstein and Warren, '62); also, rabbit blastocysts have been shown to take up the drug when administered to the pregnant female (Fabro and Sieber, '69). If the caffeine molecule were incorporated into DNA, there is a possibility, however slight, that it could give rise to an abnormal protein important in health, and heritable as well.

Although early reports in the German literature were negative (Eichler and Müggé, '32; Druckrey and Schreiber, '38; Stieve, '38), the teratogenicity of caffeine in mice was restudied by Nishimura and Nakai ('60). They found embryonic deaths and malformations consisting of limb-reduction deformities, cleft palate, and subcutaneous hematomas, after single ip injections of 250 mg/kg. In a 50-kg person this dose represents 100 cups of coffee with 125 mg of caffeine per cup (125 ml). Subsequently, results similar to those in mice were observed in chickens, rats, and rabbits (table 1). Differences in response were seen in various stocks of mice and rats. Cataracts were observed following direct fetal injection in Holtzman but not Carworth albino rats (Pitel and Lerman, '64). Teratogenicity decreased when the dose was fractionated equally 2 or 4 h apart, and the response differed depending on the route of administration and gestational age.

The typical use of caffeine by man has not been duplicated experimentally. A human being would have to drink 10–60 cups of coffee in several minutes to receive the amount given daily to rats by gastric tube. The dose required for obvious embryotoxicity in mice was 100 mg/kg (equivalent to 40 cups of coffee), with one exception: 70–75% resorptions but no malformations were found in BALB/c mice given 50–75 mg/kg/day from the 5th through the 18th days of gestation (Bertrand et al., '70).

Altogether, these reports suggest little teratogenic danger to man, and indeed no human malformation has, to my knowledge, been attributed to caffeine. Perhaps this is because man is protected by rapid metabolism of caffeine with only 1% excreted unchanged (Axelrod and Reichenthal, '53). Further experimental studies might explore species and strain differences in caffeine metabolism to seek an explanation for variations in teratogenicity.

Even more contested is the capacity of caffeine to alter genes and break chromosomes. Genetic or chromosomal changes following exposure to the drug have been seen in bacteria, fungi, and higher plants (reviewed by Fishbein et al., '70). Mutagenic action in Drosophila is still controversial (Bateman, '69). Caffeine in mice was associated with chromosomal rearrangements in spermatozoa (Adler and Rohrborn, '69); but only one report suggested dominant lethal effects (Kuhlmann et al., '68). Two larger studies of this type were negative (Adler, '69; Epstein et al., '70). When caffeine was added to human lymphocyte cultures the effects were delayed mitosis (Timson, '70) and excessive chromatid breaks (Ostertag and Greif, '67). To date, however, there are no reports of chromosomal studies of cells from heavy caffeine users.

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TABLE 1

Studies of caffeine as a teratogen, 1960–1972

Reference	Animal	Protocol: Dose (mg/kg)	Protocol: Frequency	Protocol: Route	Protocol: Days of gestation	Results
Nishimura and Nakai, '60	SMA mice	250	1	ip	7–15	d 7–12: embryonic death d 10–14: 18–43% malformations (digital defects, cleft palate, hematoma)
Kowertz et al., '62	Chicken	2 mg	(per embryo)	yolk sac	9	embryonic death; epicardial hemorrhage
Knoche and König, '64	NMRI mice	50	daily	oral	from 14 d before conception to term	10% fetal death vs. 6% in controls; 7/757 malformed vs. 0/747 in controls (3 cleft palate, 4 other facial or skull)
Pitel and Lerman, '64	Carworth rats	25–300 μg	(per fetus)	Fetal injection	16, 17, or 18	Limb defects, hemorrhage, 60% fetal death (no controls); 1/12 with cataract
	Holtzman rats	50	(per fetus)	Fetal injection	17	Limb defects, hemorrhage, 69% fetal death vs. 69% in controls; 11/17 with cataract
Bertrand et al., '65	BALB/c mice	50	daily	oral	6–18	1 malformation (adactyly) in 38 fetuses vs. 0/1000 in controls
	Swiss mice	50	daily	oral	6–18	no malformations in 116 fetuses
	Wistar rats	75–150	daily	oral	from 8 d before conception to term	dose (mg/kg): 0, 75, 100, 125, 150 resorption (%): 15, 23*, 33, 58, 62 limb reduction (%): 0.1, 3.5, 7.9, 20, 20*
Groupe d'étude . . . , '69	Swiss mice	75	daily	oral	5–sacrifice	8/439 malformations vs. 5/1022 in controls

						Malformation (%) Exp.	Malformation (%) Control	Resorption (%) Exp.	Resorption (%) Control
Fujii et al., '69	ICR-JCL mice	200	1	sc	12	21	0	14	7
		200	1	ip	12	18	1	5	1
		100	2 (2 h apart)	ip	12	7	0	4	0
		100	2 (4 h apart)	ip	12	6	0	4	0
						(cleft palate, hematoma, clubfoot, digital reduction)			
Snigorska and Bartel, '70	White mice	250–800	1	parenteral	7–14	d 7–8: resorptions dominated d 9–14: malformations dominated (maxillary defects, hematomas, limb reductions)			
Bertrand et al., '70	BALB/c mice	50, 75	daily	oral	5–18	75% resorption; 0% ectrodactyly			
	Swiss mice	50, 75	daily	oral	5–18	0% resorption; 0% ectrodactyly			
	4 breeds of rats	25, 125	daily	oral	2–15	all normal at low dose, malformations in some breeds at high dose			
	Rabbits	100	daily	oral	1–15	6 malformations (absent thumbs) in 64 fetuses			
Fujii and Nishimura, '72	Sprague-Dawley rats	180, 330	daily	oral	1–21	resorptions, edema, low birth weight with dose-response. At high dose, cranial and sacral defects and intraocular hemorrhage			
		300	daily	oral	1–7	low birth weight			
		300	daily	oral	8–14	28% resorption vs. 6% in controls; 14% malformations (esophagectopy) vs. 1% in controls			
		300	daily	oral	15–21	96% edema vs. 0% in controls; increased normoblasts			

LITERATURE CITED

Adler, I.-D. 1969 Does caffeine induce dominant lethal mutations in mice? Humangenetik, *7*: 137–148.

Adler, I.-D., and G. Rohrborn 1969 Cytogenetic investigation of meiotic chromosomes of male mice after chronic caffeine treatment. Humangenetik, *8*: 81–85.

Axelrod, J., and J. Reichenthal 1953 The fate of caffeine in man and a method for its estimation in biological material. J. Pharmacol. Exp. Ther., *107*: 519–523.

Bateman, A. J. 1969 A storm in a coffee cup. Mutat. Res., *7*: 475–478.

Bertrand, M., F. Schwam, A. Frandon, A. Vagne and J. Alary 1965. Sur un effet tératogène systématique et spécifique de la caféine chez les rongeurs. C. R. Soc. Biol., *159*: 2199–2201.

Bertrand, M., J. Girod and M. F. Rigaud 1970 Ectrodactylie provoquée par la caféine chez les rongeurs. Rôle des facteurs spécifiques et génétiques. C. R. Soc. Biol., *164*: 1488–1489.

Druckrey, H., and E. Schreiber 1938 Die Wirkung des Coffeins auf die Zelliteilung und das Wachstum. Naunyn Schmiedeberg Arch. Exp. Path. Pharmakol., *188*: 208–214.

Eichler, O., and H. Mügge 1932 Zur Frage der Schädlichkeit des Coffeins bei chronischer Zufuhr. Naunyn Schmiedeberg Arch. Exp. Path. Pharmakol., *168*: 89–96.

Epstein, S. S., W. Bass, E. Arnold and Y. Bishop 1970 The failure of caffeine to induce mutagenic effects or to synergize the effects of known mutagens in mice. Food Cosmet. Toxi., *8*: 381–401.

Fabro, S., and S. M. Sieber 1969 Caffeine and nicotine penetrate the pre-implantation blastocyst. Nature, *223*: 410–411.

Fishbein, L., W. G. Flamm and H. L. Falk 1970 Chemical Mutagens. Environmental Effects on Biological Systems. Academic Press, New York.

Fujii, T., and H. Nishimura 1972 Adverse effects of prolonged administration of caffeine on rat fetus. Toxi. Appl. Pharmacol., *22*: 449–457.

Fujii, T., H. Sasaki and H. Nishimura 1969 Teratogenicity of caffeine in mice related to its mode of administration. Jap. J. Pharmacol., *19*: 134–138.

Goldstein, A., and R. Warren 1962 Passage of caffeine into human gonadal and fetal tissue. Biochem. Pharmacol., *11*: 166–168.

Group d'étude des risques tératogènes 1969 Tératogénèse expérimentale. Etude de la caféine chez la souris. Thérapie, *24*: 575–580.

Knoche, C., and J. König 1964 Zur pränatalen Toxizität von Diphenylpyralin-8-chlor-theophyllinat unter Berücksichtigung von Erfahrungen mit Thalidomid und Coffein. Arzneimittelforschung, *14*: 415–424.

Kowertz, M. J., E. W. Lowrance and L. C. Forsythe 1962 Cardiac change in chick embryos following single injections of caffeine. Anat. Rec., *142*: 313–314 (abst.).

Kuhlmann, W., H.-G. Fromme, E.-M. Heege and W. Ostertag 1968 The mutagenic action of caffeine in higher organisms. Cancer Res., *28*: 2375–2380.

Nishimura, H., and K. Nakai 1960 Congenital malformations in offspring of mice treated with caffeine. Proc. Soc. Exp. Biol. Med., *104*: 140–142.

Ostertag, W., and B. J. Greif 1967 Die Erzeugung von Chromatidenbrüchen durch Coffein in Leukocytenkulturen des Menschen. Humangenetik, *3*: 282–294.

Pitel, M., and S. Lerman 1964 Further studies on the effects of intrauterine vasoconstrictors on the fetal rat lens. Am. J. Ophth., *58*: 464–470.

Snigorska, B., and H. Bartel 1970 Studies on the teratogenic influence of caffeine on the fetuses of white mice. Folia Morph., *29*: 353–363.

Stieve, H. 1938 Organschädigungen durch Kaffee und Coffein. Z. Mikr. Anat. Forsch., *43*: 509–557.

Timson, J. 1970 The effect of caffeine on the mitosis of human lymphocytes in culture. Br. J. Pharmacol., *38*: 731–734.

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Influence of Caffeine-containing Beverages on the Growth, Food Utilization and Plasma Lipids of the Rat [1]

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ABSTRACT It has been suggested that coffee-drinking may be a factor in the causation of cardiovascular disease, and tea drinking a factor in its prevention. In the present study, freeze-dried tea, decaffeinated coffee, coffee and pure caffeine were fed to rats, for 54 days, in a starch-based diet devoid of recognized atherogenic agents. None of the dietary supplements affected growth rate, the efficiency of food conversion or the weights of the organs. Changes produced in the plasma lipid values were proportional to the caffeine content of the diets. With increasing intake of caffeine, plasma cholesterol and phospholipid concentrations rose, while the triglyceride concentration fell. Feeding sucrose affected the plasma lipids in the opposite direction; triglycerides were increased whereas cholesterol and phospholipids were unaltered. This difference in response is attributed to a difference in the rate of lipid synthesis in the liver. The activity of pyruvate kinase, which was used as an index of lipogenesis, was unaffected by coffee, but was doubled by the substitution of sucrose for starch in the diet. Results are discussed briefly in relation to current views on the role of plasma lipids in atherogenesis.

Considerable interest has been shown in the possible role of caffeine-containing beverages in the etiology of cardiovascular disease. In a longitudinal study of some 2000 subjects, Paul et al. (1) found a positive relationship between coffee consumption and the development of coronary disease. Yudkin and Roddy (2) also reported that patients with myocardial infarction drank more tea and coffee than did an age-matched control group, but statistical analyses revealed that the association of the disease with the consumption of tea and coffee was due solely to the sugar taken with the beverages (3).

In the few experiments conducted on laboratory animals, diets containing cholesterol have been used. In the rabbit, both tea and coffee were found to reduce serum cholesterol levels and the severity of the cholesterol-induced atheromatosis (4, 5); in the rat, coffee ingestion promoted a rise in serum cholesterol, phospholipid and triglyceride concentrations, and tea a significant fall in these lipid fractions (6).

In the experiments reported here, the effect of caffeine-containing beverages on the plasma lipids of the rat was studied with a starch-based diet devoid of cholesterol. At the same time, a comparison was made between the effects on lipid metabolism of adding coffee to this diet, and replacing the starch with sucrose.

EXPERIMENTAL

The investigation was conducted in two parts. In the first part (exp. 1), the influence of dietary caffeine on growth and blood plasma lipids of the rat was determined. In the second part, a comparison was made between the effects of coffee and sucrose on plasma lipid patterns (exp. 2) and on the activity of hepatic pyruvate kinase (exp. 3), an enzyme believed to have a role in the control of lipid synthesis (7).

Experiment 1. Male rats of the Sprague-Dawley strain, from nine litters (each litter containing six or more males), were fed stock diet 41B [2] (8) until they attained an individual body weight approaching 120 g. The five pups in each litter, which during this time had remained closest in body weight, were assigned to five different dietary treatments, and transferred from stock diet to their experimental diets when they had reached 120 g.

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[1] Supported in part by a grant from General Foods.
[2] Haygates & Company, Bugbrooke Mills, Northampton, England.

Within any litter, all five animals began consuming the experimental diets within a few days of each other.

One rat from each litter received the control diet;[3] the remaining four rats in each litter were fed the control diet supplemented with coffee,[4] decaffeinated coffee,[5] tea or pure caffeine. The coffees were prepared by a standard method, then freeze-dried.[6] One cup (200 ml) of beverage contained 2.4 g coffee solids. The freeze-dried tea was a commercial product, one cup of which contained 1.0 g solids. The dried coffees and tea were added to the control diet at a concentration of 2.3 and 1.0 g/100 g diet, respectively. The fifth diet was supplemented with 140 mg pure caffeine per 100 g diet, an amount equal to the caffeine content of the supplement of dried coffee.

The amounts of the supplements consumed by the rats corresponded to a daily consumption of 12 cups of beverage by a 70-kg man. The calculation makes use of metabolic body weights (kilogram $^{0.75}$) and is based on an intake of 16 g of diet/day by a 200-g rat at the midpoint of the experiment.

The rats were housed under thermostatic conditions (22 ± 1°) in individual screen-bottom wire-mesh cages. They were weighed after 4 days, and thereafter at weekly intervals. Food consumption was measured on the days on which the animals were weighed.

After 54 days, the rats were fasted overnight then killed under ether anesthesia. Ten milliliters of blood were collected from the heart in a heparinized tube; the liver, kidneys, spleen, heart and adrenals were excised and weighed.

The total cholesterol concentration of the plasma was measured by the method of Zlatkis et al. (10) as modified by McIntyre and Ralston (11). Plasma phospholipids were determined by the method of Zilversmit and Davis (12) and triglycerides by the micromethod of Van Handel and Zilversmit (13).

The Wilcoxon matched-pairs signed-ranks test (14) was used to establish the significance of differences between the lipid values resulting from the various dietary treatments.

Experiment 2. The strain of rat, and the methods of selection and management used in experiments 2 and 3 were as described above.

Three rats from each of eight litters were transferred from stock to experimental diets when they weighed 140 g. Group 1 received the starch-based control diet, and group 2 the coffee-supplemented diet of experiment 1. In the diet of group 3, the starch was replaced quantitatively with sucrose.

After 54 days, the rats were killed following an overnight fast, and blood was drawn from the heart for lipid analyses.

Experiment 3. Three rats from each of six litters were fed, for 10 days only, diets of the same composition as had been used in experiment 2. The mean initial body weight of the rats in each group was approximately 80 g. Growth rate and food intakes were carefully measured. The animals were killed by a sharp blow on the head, and the livers were rapidly excised for estimation of pyruvate kinase activity.

Samples of liver were homogenized in 4 volumes of ice-cold 0.154 M KCl containing 0.66 mM EDTA. The homogenates were centrifuged at 15,000 rpm for 20 minutes. The activity of pyruvate kinase was measured in the supernatant fractions by the method of Valentine and Tanaka (15) modified to give a concentration of ADP in the reaction mixture of 2.4 mM. The reaction was followed at 340 μ in a spectrophotometer[7] at 25°.

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[3] Diet: (in grams) casein, 24.0; arachis oil, 8.0, maize starch, 60.0; mineral mixture (9), 5.0; Solka Floc (cellulose powder), 3.0. The mineral mixture contained: (in grams) sodium chloride, 32.0; calcium hydrogen orthophosphate monohydrate, 130.0; tripotassium citrate monohydrate, 125.0; magnesium sulfate pentahydrate, 30.0; ferric citrate pentahydrate, 5.0; and 0.7 g of a mixture of trace elements composed of potassium iodide, 12.0; sodium fluoride, 10.0; manganous sulfate tetrahydrate, 2.0; cuprous iodide, [illegible]; aluminum potassium sulfate, 1.0; and zinc sulfate heptahydrate, 1.0. To each kilogram of diet was added 2.25 g choline dihydrogen tartrate and 0.54 g of a mixture of vitamins of the B complex of the following composition: (in grams) inositol, 13.2; nicotinic acid, 6.0; calcium pantothenate, 6.0; p-aminobenzoic acid, 4.5; thiamine·HCl, 0.3; riboflavin, 1.8; pyridoxine·HCl, 0.48; biotin, 0.012; and folic acid, 0.06. In addition, each rat received, weekly, an oral dose of 0.1 ml arachis oil containing 300 IU vitamin A, 20 IU vitamin D_2, 3 mg α-tocopheryl acetate and 0.1 mg menaphthone.

[4] Maxwell House.

[5] Sanka.

[6] The freeze-dried coffees and tea were kindly prepared by Affined Foods.

[7] Unicam S. P. 800B, Unicam Instruments, Ltd., England.

RESULTS

Experiment 1. All dietary supplements caused an initial reduction in food consumption and in growth, coffee and caffeine having the most marked effect (table 1). From days 5 to 53 no notable differences in food consumption or in growth rates were observed. Thus, when the animals were accustomed to their diets, the efficiency of food utilization was essentially the same for all groups.

The differences between the body weights of the groups fed the various supplemented diets and those of the controls persisted from day 4 to the end of the experiment (day 54). Consequently, final body weights were significantly reduced ($P = 0.025$ or less). In the case of the rats which received pure caffeine, the difference was slightly increased, so that, on day 54, their body weights were 10% lower than those of their controls (table 2).

None of the dietary supplements had any effect on the weights of the organs (table 2). The small differences that were observed could be accounted for by differences in final body weights, since the weights of the viscera are related to body weight.

Table 3 summarizes the results of analyses for lipids in the plasma of the rats.

Decaffeinated coffee had no effect on plasma lipids. As the caffeine content of the diet was increased, the values for plasma cholesterol and phospholipids rose, the largest amounts of caffeine (the coffee and caffeine groups) producing statistically significant changes. The values for plasma triglycerides for all the groups receiving dietary supplements, however, were lower than that for the controls, the differences being statistically significant for rats fed tea and caffeine.

Experiment 2. The addition of coffee to the starch-based diet affected the pattern of plasma lipids of the rat differently from the change brought about by replacing the dietary starch with sucrose (table 4, fig. 1).

In experiment 1, feeding coffee produced a significant rise in the plasma cholesterol and phospholipid fractions, but did not alter the concentration of triglycerides. Conversely, the plasma triglyceride concentration rose following the incorporation of

TABLE 1

Influence of dietary supplements of tea, decaffeinated coffee, coffee and caffeine on the rate of growth and on the efficiency of food conversion of young rats (nine rats per group)

Dietary supplement	Initial body wt	Wt after 4 days	Days 5 to 53		
			Wt gain	Mean daily food intake	Wt gain per 100 g diet
	g	g	g	g	g
None	121	147	181	19.1	19.7
Sanka	120	134	179	19.2	19.4
Tea	121	138	184	18.6	20.6
Coffee	121	122	188	18.1	21.6
Caffeine	121	123	174	17.6	20.6

TABLE 2

Mean values for organ weights of control rats, and rats fed tea, decaffeinated coffee, coffee or caffeine for 54 days (nine rats per group)

Dietary supplement	Final body wt	Liver	Kidneys	Heart	Spleen	Adrenals
	g	g	g	g	g	mg
None	321	8.29	2.49	1.26	0.67	58
Sanka	302	8.03	2.41	1.15	0.58	51
Tea	313	8.17	2.47	1.16	0.60	55
Coffee	298	8.12	2.47	1.09	0.58	52
Caffeine	289	8.08	2.22	1.12	0.54	53

TABLE 3

Median values for plasma lipids of control rats, and rats fed tea, decaffeinated coffee, coffee or caffeine for 54 days (nine rats per group)

Dietary supplement	Caffeine added per 100 g diet	Total cholesterol	Phospholipids	Triglycerides
	mg/ml	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*
None	Control	85 (75–105) [1]	137 (124–149)	38 (23–65)
Sanka	4.8	85 (55–115)	143 (102–206)	34 (18–44)
Tea	77.9	95 (70–120)	159 (121–174)	29 (17–52)
Coffee	136.2	105 (80–130)	171 (126–191)	30 (19–42)
Caffeine	140.0	105 (90–140)	174 (131–190)	27 (20–41)

[1] Figures in parentheses give range of values.

Statistical analyses (*P* values)

Control versus Sanka	ns [2]	ns	ns
Control versus tea	ns	0.025	0.01
Control versus coffee	0.025	0.01	ns
Control versus caffeine	< 0.005	< 0.005	0.01
Coffee versus Sanka	0.005	0.05	ns

[2] ns = not significant.

TABLE 4

Median values for plasma lipids of rats fed starch, starch with coffee, or sucrose for 54 days (eight rats per group)

Diet	Total cholesterol	Phospholipids	Triglycerides
	mg/100 ml	*mg/100 ml*	*mg/100 ml*
Starch	59 (50–70) [1]	124 (100–139)	37 (28–39)
Starch/coffee	90 (65–122)	148 (121–183)	35 (29–45)
Sucrose	61 (50–72)	124 (105–135)	53 (42–78)

[1] Figures in parentheses give range of values.

Statistical analyses (*P* values)

Starch versus starch/coffee	0.005	0.005	ns
Starch versus sucrose	ns [2]	ns	0.005

[2] ns = not significant.

sucrose in the diet, but the cholesterol and phospholipid fractions were unaffected.

Experiment 3. It is believed that the blood plasma triglycerides may be synthesized exclusively in the liver (16). Because coffee and sucrose appeared to differ fundamentally in their influence on the plasma lipids, we sought an explanation for this difference in the metabolism of lipids in the liver. The activity of pyruvate kinase was measured in rats fed the same diets as were used in experiment 2; the results are shown in table 5.

Pyruvate kinase activity was not changed by the addition of coffee to the starch-containing diet. The replacement of starch with sucrose, however, doubled the activity of this enzyme ($P = 0.001$).

DISCUSSION

In most experiments designed to test the importance of diet in the development of cardiovascular disease, the amounts and proportions of the major serum lipid fractions have been the parameters most frequently measured.

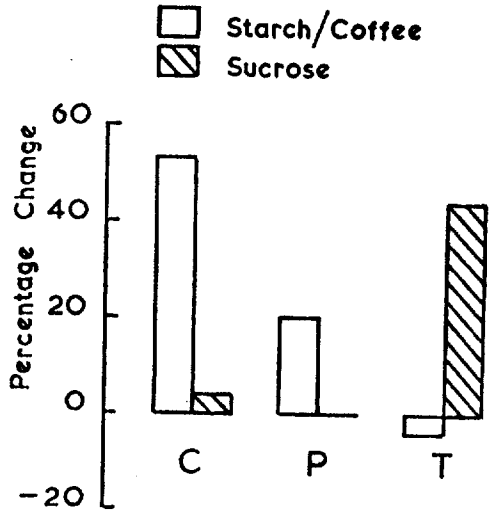

Fig. 1 Comparison of the effects on plasma cholesterol (C), phospholipids (P) and triglycerides (T) of supplementing a starch-based diet with coffee, and replacing starch with sucrose.

TABLE 5

Activity of pyruvate kinase in the livers of rats fed starch, starch with coffee, or sucrose (six rats per group)

Diet	Median	Range
Starch	7.25 [1]	6.45– 8.31
Starch/coffee	6.95	6.32–11.72
Sucrose	15.64	13.38–21.21

[1] Values of enzyme activity are given as micromoles of substrate converted per minute per gram of wet tissue.

Little and his colleagues (17, 18) in a retrospective study of the diet of male survivors of myocardial infarction, found a positive and statistically significant correlation between coffee consumption and serum lipid concentrations in their patients, although they failed to corroborate the original observation of Paul et al. (1) that coffee drinking was more common in such patients than in age-matched control subjects.

Changes in plasma lipid levels in the rat resulting from the feeding of tea and coffee have been described by Akinyanju and Yudkin (6). In a diet containing cholesterol, coffee had the effect of raising the concentrations of cholesterol, phospholipids and triglycerides, whereas tea caused a fall in all three fractions. As both these beverages contain caffeine, it was argued that caffeine could not be involved in the disturbances noted in the metabolism of the lipids.

Since the addition of cholesterol to a diet is known to raise the blood cholesterol level and suppress the synthesis of cholesterol in the liver,[8] the intervention of other dietary components in the metabolism of endogenous cholesterol might have been obscured. Furthermore, any substance in the diet that impaired the absorption of exogenous cholesterol would appear to exert a hypocholesterolemic effect.

In the present investigation, the influence of tea, decaffeinated coffee, coffee and caffeine on lipid metabolism was measured with a basic diet devoid of any of the agents commonly employed for the induction of experimental atherosclerosis. Under these conditions, the magnitude of the change in serum lipid concentrations was directly related to the caffeine content of the diet. The supplement of tea provided approximately half as much caffeine as the supplement of coffee, and caused a rise in plasma cholesterol that was half of that produced by the coffee. Decaffeinated coffee had no effect on plasma cholesterol; pure caffeine reproduced precisely the effects of coffee.

The rise in the concentration of phospholipids and the fall in the concentration of triglycerides were also in proportion to the intake of caffeine.

The changes in the plasma lipid concentrations brought about by tea and coffee in the absence of dietary cholesterol are therefore very different from those noted when cholesterol was fed (6). The apparent hypocholesterolemic effect shown by tea in the latter case may have been due to the impairment of cholesterol absorption by some component of tea, probably the tannins. The elevation of the plasma triglyceride level by coffee, but not by decaffeinated coffee, in the presence of exogenous cholesterol would appear to be an effect of caffeine which was not in evidence when cholesterol was eliminated from the diet.

There is a considerable body of evidence in support of the claim that sucrose may

[8] Gould, R. G., and C. B. Taylor 1950 Effect of dietary cholesterol on hepatic cholesterol synthesis. Federation Proc., 9: 179 (abstract).

be an important dietary factor in the development of coronary heart disease (19). Much of the sucrose is taken in association with tea and coffee, and the suggestion has been made (3) that the relationship found between the quantities of tea and coffee habitually consumed and the occurrence of heart disease may be due to the sugar consumed in these beverages.

Rats which have been fed sucrose show a rise in the plasma concentration of triglycerides, but no significant alteration in either total cholesterol or phospholipids (20). The change in the pattern of the plasma lipids following the administration of caffeine-containing beverages appeared, therefore, to be the converse of that resulting from sucrose feeding. This conclusion was confirmed in experiment 2.

It is probable that the disparity in the effects of dietary sucrose and caffeine on the plasma lipids results from differences in the rates of synthesis of the triglycerides in the liver. In experiment 3, the activity of hepatic pyruvate kinase was used as an index of lipogenesis. The addition of coffee to a starch-based diet had no effect on enzyme activity, whereas activity was doubled by substituting sucrose for starch in the diet.

The conclusions to be reached from these experiments with regard to the possible role of caffeine-containing beverages in the etiology of coronary heart disease must be based on the significance attached to alterations in the various plasma lipid fractions.

For many years, it has been recognized that patients with heart disease frequently have an elevated plasma cholesterol concentration (21). The increase in the plasma cholesterol of the rat resulting from the ingestion of tea and coffee might therefore imply that they are potentially harmful constituents of the diet. Recent reports have suggested, however, that a rise in the plasma triglyceride fraction is the more characteristic change encountered in atherosclerosis and coronary heart disease (22), in which case tea and coffee-drinking would be absolved.

The final judgment on these beverages must therefore be suspended until the mechanisms involved in the process of atherogenesis are clearly understood.

LITERATURE CITED

1. Paul, O., M. H. Lepper, W. H. Phelan, G. W. Dupertuis, A. MacMillan, H. McKean and H. Park 1963 A longitudinal study of coronary heart disease. Circulation, *28:* 20.
2. Yudkin, J., and J. Roddy 1964 Levels of dietary sucrose in patients with occlusive atherosclerotic disease. Lancet, *ii:* 6.
3. Yudkin, J., and J. Morland 1967 Sugar intake and myocardial infarction. Amer. J. Clin. Nutr., *20:* 503.
4. Heyden, Von S., and J. R. Rüttner 1966 Die Beeinflussung des Cholesterinspiegels durch Coffein im Tierversuch. Pathol. Microbiol., *29:* 291.
5. Young, W., R. L. Hotovec and A. G. Romero 1967 Tea and atherosclerosis. Nature, *216:* 1015.
6. Akinyanju, P., and J. Yudkin 1967 Effect of coffee and tea on serum lipids in the rat. Nature, *214:* 426.
7. Krebs, H. A., and L. V. Eggleston 1965 The role of pyruvate kinase in the regulation of gluconeogenesis. Biochem. J., *94:* 3c.
8. Bruce, H. M., and A. S. Parkes 1947 Feeding and breeding of laboratory animals. III. Observations on the feeding of guinea-pigs. J. Hyg., *45:* 70.
9. de Loureiro, J. A. 1931 Quelques points importants a considérer dans l'étude experimentale de l'alimentation. Arch. Patol., Lisboa, *3:* 72.
10. Zlatkis, A., B. Zak and A. J. Boyle 1953 A new method for the direct determination of serum cholesterol. J. Lab. Clin. Med., *41:* 486.
11. MacIntyre, I., and M. Ralston 1954 Direct determination of serum cholesterol. Biochem. J., *56:* iii.
12. Zilversmit, D. B., and A. K. Davis 1950 Microdetermination of plasma phospholipids by trichloroacetic acid precipitation. J. Lab. Clin. Med., *35:* 155.
13. Van Handel, E., and D. B. Zilversmit 1957 Micromethod for the direct determination of serum triglycerides. J. Lab. Clin. Med., *50:* 152.
14. Siegel, S. 1956 Nonparametric Statistics: For the Behavioural Sciences. McGraw-Hill Book Company, New York.
15. Valentine, W. N., and K. R. Tanaka 1966 Pyruvate kinase: clinical aspects. In: Methods in Enzymology, vol. 9, Carbohydrate Metabolism, ed., W. A. Wood. Academic Press, New York.
16. Swell, L., and C. R. Treadwell 1963 Interrelationships of lipids in blood and tissues. In: Atherosclerosis and Its Origin, eds., M. Sandler and G. H. Bourne. Academic Press, New York.
17. Little, J. A., H. M. Shanoff, A. Csima, S. E. Redmond and R. Yano 1965 Diet and serum lipids in male survivors of myocardial infarction. Lancet, *i:* 933.
18. Little, J. A., H. M. Shanoff, A. Csima and R. Yano 1966 Coffee and serum lipids in coronary heart disease. Lancet, *i:* 732.

19. Yudkin, J. 1967 Why blame sugar? Chem. Ind. 1464.
20. Akinyanju, P. A., and J. Yudkin 1967 Effects of dietary carbohydrates on the serum lipids of the rat. Proc. Nutr. Soc. England, Scotland, 26: xxxi.
21. Keys, A., H. L. Taylor, H. Blackburn, J. Brozek, J. T. Anderson and E. Simonson 1963 Coronary heart disease among Minnesota business and professional men followed fifteen years. Circulation, 28: 381.
22. National Research Council 1966 Dietary Fat and Human Health, publ. no. 1147. National Academy of Sciences — National Research Council, Washington, D. C.

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Failure of Dexedrine and Caffeine as Practical Antagonists of the Depressant Effect of Ethyl Alcohol in Man[1]

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ALTHOUGH there has been some work on pharmacologic antagonists of alcohol in animals (1), and clinical reports of the sobering effect of such diverse agents as carbon dioxide inhalation (2) and insulin and glucose infusions (3) have appeared, the literature fails to reveal any study of the possible antagonizing effect of cerebral stimulants on milder degrees of alcohol intoxication in man. It is conceivable that situations may arise in which it would be advantageous to consume considerable quantities of alcohol without suffering the depression of nervous function usually produced thereby. The present study was undertaken to assess the practical value of two drugs commonly used for cerebral stimulation for this purpose. Since the objective was to find a practical antagonist to alcohol, the dosages employed were in the ordinary therapeutic range, while the tests of nervous function were those in which previous experience had shown failure to occur at blood alcohol concentrations close to those associated with mildly incapacitating intoxication.

Methods

Subjects: Six subjects were used, members of the staff and student body of the Medical School. They represented a fairly homogeneous group in that social and educational factors were reasonably uniform, while all could be classed as occasional or moderate users of alcohol. Four were in the third decade of life and two in the fifth. All knew the purpose of the experiment. Since all the drugs used are easily recognized by their subjective effects by the initiate, no attempt at substitution of placebos was made. Because of the fairly high doses of alcohol employed, subjects were kept under close observation until their behavior had returned to normal, an essential precaution in this type of experimentation.

Administration of alcohol: Alcohol was administered by mouth, the choice of beverage between grain alcohol, Bourbon whisky and Scotch whisky being left to the subject. Each dose was equal to 0.1 cc. of alcohol per pound

[1] From the Department of Medicine, Stanford University School of Medicine San Francisco.

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of body weight, diluted to about 20 per cent concentration with either plain or carbonated water, and consumed within 5 minutes. The dose was repeated at intervals of 20 minutes. Subjects were instructed to eat a substantial meal about 3 hours before the start of the experiment, and nothing thereafter. This insured a reasonably empty stomach to facilitate absorption, without the low blood sugar which might occur after a longer fast.

Administration of antagonists: The drugs tested were taken by mouth 45 minutes before the start of the experiment. The dose of dexedrine was 15 mg., that of caffeine, 300 mg. These were well tolerated by the subjects, although one of them, not used to drinking coffee, noted some unpleasant symptoms after caffeine. Since the duration of the critical part of the experiment was only about 2 hours, the dose was not repeated.

Tests of nervous system function: A battery rather than any single test was used to assess the depressant effect of alcohol on the nervous system. There is no single test that is entirely satisfactory, as the lack of good correlation between the tests used demonstrates. The tests were the following:

1. Balance on one foot with eyes closed. The subject was allowed to balance on one foot with eyes open; at a signal from him he closed his eyes and the observer started a stopclock. Moving without lifting the foot from the floor was allowed. If at the end of 15 seconds he was still upright the test was passed. If not, two further trials on that foot were allowed, and if either was passed, the same procedure was carried out with the other foot. Failure in three trials on either foot was scored as failure in the test, the all or nothing principle being used and no degrees of failure being scored. This is a variation of the test for equilibrium used in the examination of naval aviators, with which one of the authors first became familiar during World War II. Experience with this test on at least ten occasions over the past 13 years has shown the blood alcohol concentration at which failure occurred to vary between the narrow limits of 145 and 165 mg. per cc. Because of our confidence in this simple test, which shows a negligible effect of learning, we used it as the criterion for terminating the experiment.

2. Steadiness of hand. The subjects endeavored to keep a metal stylus ⅛ inch in diameter held at arm's length within a hole 3/16 inch in diameter without touching the edge of the hole. The time during which the stylus made contact was measured electrically. With the duration of the test 1 minute, a perfect score was zero, total failure 50. This test was little affected by learning, but fatigue and chance variation were found to affect performance during control experiments as much as 8 points, so that arbitrarily a drop of 15 points was taken as the index of failure.

3. Electroencephalogram. Five needle electrodes were placed in the right and left parietal and right and left occipital positions, and the mastoid as ground, and 4 channels of a Grass Model 2 or 3 electroencephalograph were used to record transoccipital, transparietal, and right and left posterior bipolar electroencephalograms. The electrodes were left in place throughout the experiment, the leads being clipped to the coat collar between tests. The dominant rhythm was counted, and since all

subjects had been chosen with a stable rhythm in the region of 10 cycles, a drop in frequency of 1 cycle in 20 per cent of the record was a reliable indicator of the effect of alcohol.

4. FUSION FREQUENCY OF FLICKER. A neon lamp with a circular electrode about 2 cm. in diameter was placed 2 meters from the subject whose chin was supported in a headrest. The lamp was actuated from the output of a Grass Model 3 stimulator, the voltage being maintained constant at full output with flash duration of 20 milliseconds. The lamp obviously flickering, frequency was increased until the subject reported fusion. Frequency was then decreased until flickering was again noted. The mean of three trials was recorded. Subjects varied greatly in the range of values obtained on control runs. Since the variation was greater in the onset of flicker than of fusion, the latter values only were finally utilized. A drop in rate of 1 cycle greater than the range shown by that particular subject in control runs was considered significant.

Experimental Procedure: All subjects were first given a control run, the tests being administered every 20 minutes for 2 hours. On subsequent days a similar control was run 45 minutes after the dose of dexedrine or caffeine. Performance on the balancing test was the same in all controls, no failures being recorded. One subject showed an increase in 4 cycles in the electroencephalogram with caffeine, another a decrease of 1 cycle with dexedrine. Hand steadiness was essentially the same in the three control runs, indicating lack of practice effect. Fusion frequency dropped significantly in three subjects after dexedrine, in one after caffeine. After these three control runs, a run with alcohol alone was done, then with alcohol and dexedrine, and finally with alcohol and caffeine.

RESULTS

The results are presented in Table 1. The blood alcohol value at which failure in each test occurred, using the arbitrary criteria of failure set forth above, was calculated from a plot of performance in the test against the blood alcohol concentration, a sample of venous blood from a cubital vein being obtained for alcohol determination (4) at the time that failure on the test of balance occurred and subsequently each 20 minutes until the test was again passed. From the curve obtained, the blood alcohol concentration at which failure in the other tests occurred could be reliably estimated. When the test was passed at the highest blood alcohol concentration achieved, this is designated by the letter P and the highest alcohol value.

The table demonstrates that in none of the subjects was there evidence of significant elevation of the concentration of alcohol in the blood at which failure on the tests occurred. Of the tests employed, the most consistent results in respect to the blood alcohol

TABLE 1.—*Blood Alcohol Concentration at which Failure on Tests Occurred with Alcohol Alone, Alcohol and 15 mg. of Dexedrine, and Alcohol and 300 mg. of Caffeine.**

Subject	*Medication*	*Balance*	*Steadiness*	*Fusion*	*EEG*
He.	Alcohol	152	138	24	170
	Alc. + Dexed.	150	168	40	95
	Alc. + Caff.	165	121	18	115
Sc.	Alcohol	90	55	P110	75
	Alc. + Dexed.	85	62	P110	70
	Alc. + Caff.	102	68	P104	48
Ca.	Alcohol	95	80	30	63
	Alc. + Dexed.	110	P121	P121	57
	Alc. + Caff.	105	83	P123	70
Co.	Alcohol	110	110	38	100
	Alc. + Dexed.	120	118	P126	123
	Alc. + Caff.	122	132	P125	92
Po.	Alcohol	73	68	P85	P85
	Alc. + Dexed.	65	55	P72	58
Ed.	Alcohol	151	92	P151	122
	Alc. + Dexed.	155	95	P163	90

* P indicates that the test was passed at the maximum blood alcohol concentration attained. The maximum concentration is shown following the letter.

concentration at which failure occurred must be credited to the simple test of balance. This test correlated fairly well with the electroencephalogram, less well with steadiness of hand, and poorly with fusion frequency.

Discussion

It is apparent from the results shown in Table 1 that neither dexedrine nor caffeine can be considered an effective practical antagonist to the depressant effect of alcohol when used in ordinary therapeutic dosage. Using the test of balance, it is true that all four subjects who received alcohol and caffeine failed at a higher blood alcohol concentration than with alcohol alone, but in no case was the increase more than 13 mg. per cc., or about 10 per cent of the total value. Since the caffeine test was the last of the series, it might even be argued that the improved performance was due to learning, or even to acquired tolerance, although we think neither of these possibilities likely. With dexedrine, the concentration at failure increased in three and decreased in three subjects, again by small amounts. In none of the other tests of nervous

function was consistent evidence of greater tolerance to alcohol recorded.

Since it was our objective to assess these drugs for practical value as alcohol antagonists, it is of little importance to calculate whether or not the small differences recorded are statistically significant; the fact remains that even if significant, an increase in tolerance of 10 per cent is not of practical value. Indeed, an improvement of the order of 50 per cent would be necessary to increase the tolerance of the less tolerant of our subjects to that of the two most tolerant.

Summary

Dexedrine and caffeine in ordinary therapeutic doses were ineffective in combating the depressant effect of alcohol on the nervous system to a practical degree in a group of normal human subjects.

REFERENCES

1. Reifenstein, E. C., Jr. Amphetamine sulphate-ethyl alcohol antagonism in the rabbit. J. Pharmacol. **69:** 298–299, 1940.
2. Robinson, L. J. and Selesnick, S. The treatment of acute alcoholism with 10 per cent carbon dioxide and 90 per cent oxygen inhalation. J. Amer. med. Ass. **105:** 1734–1738, 1935.
3. Goldfarb, W., Bowman, K. M. and Parker, S. The treatment of acute alcoholism with glucose and insulin. J. clin. Invest. **18:** 581–584, 1939.
4. Newman, E. J. and Newman, H. W. A microdiffusion method for alcohol determination. Stanford med. Bull. **11:** 96–99, 1953.

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Congenital Malformations in Offspring of Mice Treated with Caffeine.* (25757)

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Certain substances which act as mitotic poisons have been reported to have teratogenic effects in mammalian embryos(1,2). However, many chemicals present in foods, beverages or drugs have not been tested or have been insufficiently studied for possible teratogenic effects. A few studies on caffeine have been reported. Druckrey and Schreiber(3) observed cessation or irregularity in mitosis in fertilized eggs of sea urchin after these were dipped in a caffeine solution. Eichler and Mügge(4) found no deleterious effects on embryonic development when pregnant rats were injected subcutaneously with large doses of caffeine. However, continuous treatment of pregnant rabbits with high doses of caffeine resulted in occasional early embryonic death or retarded development; the newborn young appeared to have "poor resistance"(5-7). Since no congenital malformations have been reported, we have investigated the effects of high doses of caffeine injected in pregnant mice.

Methods. Approximately 100 pregnant mice of the SMA strain (originating from Japanese Nat. Inst. of Genetics) were given a single intraperitoneal injection of 1% caffeine once during 7th to 15th days of pregnancy. A dosage level of 0.25 mg/g body weight, considered to be approximately the maximum dose tolerated, was used. The animals were sacrificed near term or in mid-pregnancy and fetuses weighed, examined for anomalies, and fixed in 20% formalin solution. Fetuses obtained from 25 normal pregnant mice were used as controls.

Results. Table I shows that caffeine administration may result in embryonic death or in malformed fetuses. Incidence of embryonic death was highest for injections given during 7th-12th days, when resorption of all embryos of a litter was sometimes induced. However, any injection given between 7th-14th days resulted in some dead or macerated fetuses. Malformations were observed in 18 to 43% of fetuses when injections were given between 10th and 14th days; a single malformation was found for injections on 9th day

TABLE I. Effects of Intraperitoneal Injection of Caffeine* in Pregnant Mice Observed at Term.

Day of inj.	No. of mice	No. of complete resorptions	Young		
			Total No.	% dead	% abnormal
7	4	2	11	18	0
8	5	0	36	6	0
9	11	2	45	11	2
10	6	0	40	3	38
11	10	0	57	12	19
12	8	1	41	17	41
13	7	0	51	8	43
14	7	1	44	18	18
15	3	0	24	0	0
Uninjected controls	25	0	225	2	0

* 1% aqueous solution, 0.25 mg/g body wt.

* We are indebted to Dr. M. M. Nelson, Dept. of Anatomy, University of California, San Francisco, for advice and aid in preparation of manuscript.

TABLE II. Number and Types of Malformations Observed in Offspring of Pregnant Mice Given Intraperitoneal Injections of Caffeine.*

Day of inj.	Abnormal young No.	No. of young with specific malformations in: Digits Fore	Hind	Joints†	Palate	Hematoma	Other
9	1				1		
10	15	3	10	3	2		1
11	11	8	8		1	1	
12	17	14	9	4	10	12	
13	22	14	7	2	9	15	1
14	8	1	8	2		4	

* 1% aqueous solution, 0.25 mg/g body wt.
† This column includes clubfoot as well as joint malformations.

of pregnancy. No significant effects on embryonic body weights were observed. All placentae were normal in size, shape, and color and histological study of representative placentae for each litter did not reveal any morphological changes.

The malformations observed were predominantly those of skeletal system, especially digital defects and cleft palate (Table II). Digital defects included angulation, brachydactylism, syndactylism, adactylism, and polydactylism. The critical period for adactylism in forefeet was 10th-13th days and in hindfeet 11th-14th days. Clubfoot (*pes varus*) and joint malformations were also observed. Cleft palate was induced by injections between 9th and 13th days with the highest incidence observed for injections given on 12th or 13th day. All cleft palates were bilateral and complete but of varying widths. One cleft palate was accompanied by harelip, in which a cleft of left premaxillary bone as well as corresponding upper lip was found. Cleft palates were often associated with hematomas in subcutaneous or deeper layers of upper and lower jaws. Adactylism and brachydactylism were also usually associated with superficial hematomas. In some cases these were comparatively small, localized hematomas accompanied by congestion of adjacent blood vessels in subcutaneous tissues. In other cases large hematomas were present in deeper tissues and were often associated with absence, hypoplasia or deformity of phalanges and metacarpal bones, and disturbances of muscle development.

Subcutaneous hematomas were observed when an injection was given on or after 11th day (Table II). When pregnant mice were sacrificed in mid-pregnancy, hematomas could be recognized as early as 12 hours after injection but were most frequent in older embryos of 15-16 days fetal age. In earlier stages hematomas were usually confined to fore and hind feet but in later stages they occurred in other areas such as maxilla, mandible and tip of tail. Number and size of hematomas increased with length of time following injection. Although there appears to be a correlation between hematomas and certain malformations, other defects observed in this study such as malformed elbow joints, *pes varus* and polydactylism were not accompanied by hematomas. The association of digital deformities with hematomas resulting from caffeine administration appears to be similar to results obtained with deficiencies of linoleic acid(8) and pantothenic acid(9,10).

The placental transfer of caffeine observed in rabbits and dogs(11) and the lack of macroscopic or microscopic changes observed in the placenta in this study permit the suggestion that caffeine may have directly affected the developing embryo.

Summary. Pregnant mice were given single intraperitoneal injections of caffeine on different days of gestation. When injections were administered between 9th and 14th days, malformations, predominantly in the skeletal system, were observed. Superficial hematomas observed in vicinity of anomalies may have been concerned in development of some of these malformations. No macroscopic or microscopic changes were observed in placentae of malformed young.

1. Nishimura, H., *Saishin Igaku (Jap.)*, 1956, vVII, 2783.

2. Kalter, H., Warkany, J., *Physiol. Rev.*, 1959, v39, 69.

3. Druckrey, N., Schreiber, E., *Arch. Exp. Pathol. Pharmakol. Naunyn-Schmiedeberg's*, 1938, v188, 208.

4. Eichler, O., Mügge, H., *ibid.*, 1932, v168, 89.

5. Stieve, H., *Med. Welt*, 1929, v3, 1133, 1173.

6. ———, *Z. Mikro.-Anat. Forsch.*, 1938, v43, 509.

7. Fischer, H., *ibid.*, 1940, v47, 275.

8. Martinet, M., *Ann. Med. Paris*, 1952, v53, 286.

9. Giroud, A., Lefébvres, J., Prost, H., Dupuis, R., *J. Embryol. Exp. Morph.*, 1955, v3, 1.

10. Nelson, M. M., Wright, H. V., Baird, C. D. C., Evans, H. M., *J. Nutrition*, 1957, v62, 395.

11. Fabre, R., Regnier, M., *C. R. Soc. Biol. Paris*, 1934, v116, 155.

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SHORT COMMUNICATION

The mutagenic activity of caffeine in man

It has been suggested that caffeine (1,3,7-trimethylxanthine) a purine, might be mutagenic in man[7]. The mutagenic activity of caffeine has been demonstrated in bacteria[3,4,6,14], ascomycetes[5], Ophiostoma[19], Drosophila[1], and higher plants[10,11]. The results with mice are as yet inconclusive[2,13]. Caffeine interacts with DNA in lowering the melting temperature of DNA[18].

Since tissue-culture cells—mainly HeLa cells—proved to be the best available material for studying induced chromosome breakage in human cells, caffeine was tested in HeLa cells. Later on the studies were extended to leukocyte short-term cultures. The results were essentially the same as those obtained with HeLa cells.

HeLa cells were cultured[12], and the chromosomal analysis carried out as described by HAM AND PUCK[8]. Colchicine was not used. The leukocyte cultures were grown and analyzed following usual methods with some minor changes.

HeLa cells were treated for 1 h with varying concentrations of caffeine dissolved in Eagle's medium. Medium without caffeine was then added after twice washing the cells, and the cells were incubated for another 24 h. Chromosomal analysis was then carried out. A period of 24 h was chosen since after 24 h the maximum amount of breakage with actinomycin D (ref. 17) was found. We are now studying the maximum effectivity of caffeine during the cell division cycle.

The results are shown in Table I and Fig. 1.

As can be seen, caffeine is effective in breaking chromosomes of human cells in culture. The amount of breakage is almost linear with dose and extrapolates to control values at 0 dose. At higher doses (10 mg/ml) the effects seem to be greater than linear. The type of chromosomal aberrations produced by caffeine is distinctly different from that produced in HeLa cells or leukocyte cultures by acridine dyes, actinomycin[17], by ethyleneimine, and mitomycin C (refs. 15, 16), or by ethoxycaffeine[9]. The latter agents produce a high percentage of translocation figures (actinomycin D, proflavin) and heterochromatic "cross-figures" (ethyleneimine, mitomycin C and ethoxycaffeine), but the production of translocations by caffeine is fairly low, though translocation figures are present in a few cases. Recent results show that caffeine acts only if it is

TABLE I

NUMBER OF BREAKS INDUCED BY CAFFEINE PER CHROMOSOME
Chromosome analysis 24 h after 1-h exposure.

Caffeine (µg/ml)	Number of chromosomes	Chromatid + isolocus breaks	Number per chromosome	Translocations	Dicentrics	Rings
0	4187	4	0.0009		–	–
500	2754	18	0.0065	–	1	–
1000	3693	47	0.0128	*	2	–
2000	4361	111	0.0250	1	–	–
3000	2663	106	0.0398	1	2	
5000	5666	401	0.0708	5	2	3
10000	3456	763	0.221	11	3	–

* 2 cells with 10 translocations.

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added before or during DNA replication. The majority of breaks do not rejoin, and 50 h after treatment no more breakage can be detected. Breakage can also be detected in normal diploid leukocyte cultures, although the morphology of the chromosomes was not adequate for counting purposes. Examples of the type of breakage

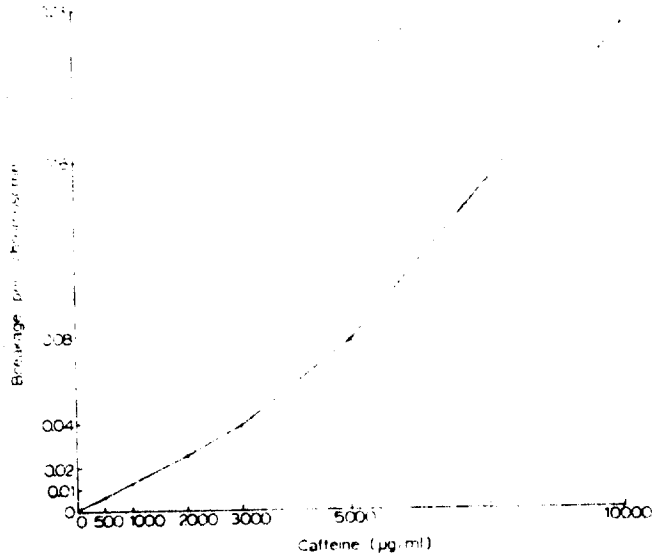


Fig. 1. Breakage induced by caffeine in HeLa cells. Dose dependence.

Fig. 2. Breakage and deletions due to caffeine in HeLa cells.

and translocations produced by caffeine in HeLa cells are shown in Figs. 2 and 3. The inhibition of cell growth by caffeine is shown in Fig. 4.

Extrapolating the effects of caffeine on HeLa cells (assuming that normal diploid chromosomes are equally breakable), we can estimate that 1 mg/ml caffeine added to the medium induces an average of 0.0125 breaks per two chromatids. In the two daughter cells there would be 0.006 breaks per chromosome, and approx. 0.3 breaks per cell with 46 chromosomes. Estimating the number of human genes

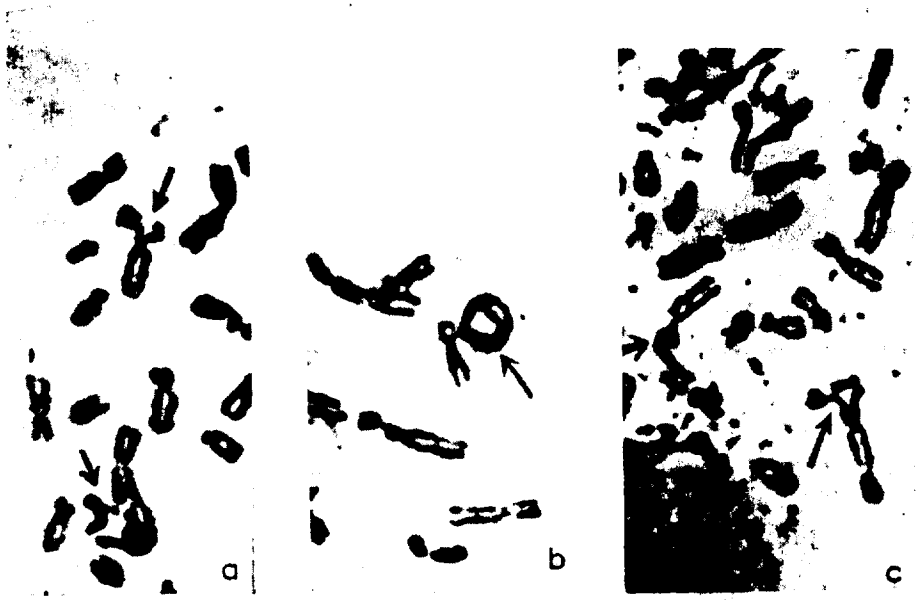
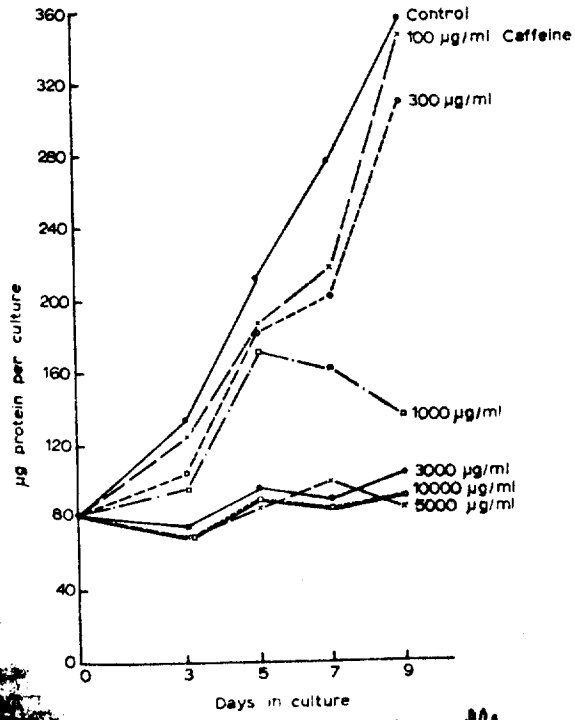


Fig. 3. Chromosomal aberrations induced by caffeine: a, translocation figures; b, ring; c, translocation figures and breakage.



Growth inhibition by caffeine, HeLa cells.

$2 \cdot 10^5$ (ref. 7), this would mean that 1 mg/ml caffeine might produce an average of $1.5 \cdot 10^{-5}$ breaks per gene. Since the production of breakage seems to be linearly dependant on dose, it is possible that the much lower doses received by man in drinking coffee or tea are mutagenic as well (proportional to the dose). Using the calculations given by GOLDSTEIN (ref. 7, p. 180–181) and assuming that caffeine is equally mutagenic in human testicular tissue, rough calculations place the amount of damage (breakage) in the neighbourhood of the "natural mutation rate".

Further studies are in progress to determine the effects of theobromine and theophylline.

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1 ANDREW, L. E., The mutagenic activity of caffeine in Drosophila. *Am. Naturalist*, 43 (1959) 135–138.
2 CATTANACH, B. M., Genetical effects of caffeine in mice. *Z. Vererbungslehre*, 93 (1962) 215–16.
3 DEMEREC, M., G. BERTANI AND J. FLINT, A survey of chemicals for mutagenic action on *E. coli*. *Am. Naturalist*, 85 (1951) 119–136.
4 DEMEREC, M., E. M. WITKIN, B. W. CATLIN, J. FLINT, W. L. BELSER, C. DISSOSWAY, F. L. KENNEDY, N. C. MEYER AND A. SCHWARTZ, The gene. *Carnegie Inst. Wash. Publ.*, 49 (1959) 144–151.
5 FRIES, N., The production of mutations by caffeine. *Hereditas*, 36 (1950) 134–149.
6 GEZELIUS, K. AND N. FRIES, Phage-resistant mutants induced in *Escherichia coli* by caffeine. *Hereditas*, 38 (1952) 112–114.
7 GOLDSTEIN, A., Mutagens currently of potential significance to man and other species mutations. In W. J. SCHULL, *Second Conference on Genetics*, The University of Michigan Press, Ann Arbor 1962, p. 167–242.
8 HAM, R. G. AND T. T. PUCK, Quantitative colonial growth of isolated mammalian cells. In S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. 5, Academic Press, New York, 1962, p. 90–118.
9 JACKSON, J. F., Chromosome aberrations in cultured human leukocytes treated with 8-ethoxycaffeine. *J. Cell Biol.*, 22 (1964) 291–293.
10 KIHLMAN, B., A survey of purine derivatives as inducers of chromosome changes. *Hereditas*, 38 (1952) 115–127.
11 KIHLMAN, B. AND A. LEVAN, The cytological effect of caffeine. *Hereditas*, 35 (1949) 109–114.
12 LEVINTOW, L. AND H. EAGLE, The technique of mammalian cell culture. In S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol 5, Academic Press, New York, 1962, p. 77–89.
13 LYON, M. F., J. S. R. PHILLIPS AND A. G. SEARLE, A test for mutagenicity of caffeine in mice. *Z. Vererbungslehre*, 93 (1962) 7–13.
14 NOVICK, A., Mutagens and antimutagens. *Brookhaven Symp. Biol.*, 8 (1955) 201–215.
15 NOWELL, P. C., Mitotic inhibition and chromosome damage by mitomycin in human leukocyte cultures. *Exptl. Cell Res.*, 33 (1964) 445–449.
16 OSTERTAG, W. AND H. KERSTEN, The induction of chromosomal aberrations with mitomycin C and ethylenimine in human cells *in vitro*. To be published.
17 OSTERTAG, W. AND W. KERSTEN, The action of proflavin and actinomycin D in causing chromatid breakage in human cells. *Exptl. Cell Res.*, in the press.
18 TS'O, P. O. P., G. K. HELMKAMP AND C. SANDER, Interaction of nucleosides and related compounds with nucleic acids as indicated by the change of helix–coil transition temperature. *Proc. Nat'l. Acad. Sci. U.S.*, 48 (1962) 686–697.
19 ZELLERKORN, G., The mutagenic effect of 8-ethoxycaffein, caffein and dimethylsulfate in the Ophiostoma back-mutation test. *Hereditas*, 46 (1959) 279–311.

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51. *Human Caffeine Fatality*. R. F. Parish, C. Frick, A. B. Richards, and R. B. Forney, Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, Indiana.

It has been estimated that 5–10 g of caffeine taken orally may be fatal in man (Krantz and Carr, *Pharmacologic Principles of Medicine*, 5th Edition). However, distribution data in body tissues of fatal doses are not known. The opportunity for study of tissue distribution was presented to our laboratory in a suicidal case possibly involving caffeine. Various methods of identification and quantitation of caffeine were performed with the submitted tissues using ultraviolet and infra-red spectrum, spot tests, and thin-layer chromatography. The quantitative results obtained from the various tissues in mg of caffeine alkaloid per 100 g of tissue were: kidney, 12.96; liver, 32.90; brain, 7.47; stomach, 150.66. Comparative studies of tissue distribution levels of fatal dosages of caffeine were extended using rats. Ten male rats averaging 200 g in weight were given an oral dosage of 1 g/kg of caffeine alkaloid suspended in a 2% solution of acacia, which was kept warm to insure uniform suspension. The time of administration was recorded as well as the time of death. Immediately following death, kidney, liver, brain, and blood samples were obtained for analysis. Brain and kidney tissues were pooled for analysis whereas blood and liver analyses were performed separately. Identical means of identification and quantitation were used in determining rat tissue levels as in the suicidal case. The following results were obtained for rat tissue distribution expressed as mg of caffeine alkaloid per 100 g of tissue: kidney, .879; brain, .64; blood (average of 10 samples), 1.03; liver (average of 10 samples), 1.41. If distribution data from the rat experiment are compared to those of the suicidal case, it is apparent that the distributions of caffeine in both are in the same sequential order.

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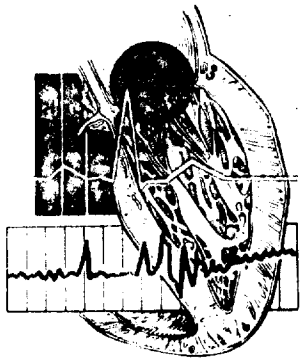

Stimulants and Coronaries

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Does the man who drinks coffee excessively increase his risk of having a coronary occlusion? Perhaps. In this study of 1,718 men, those who drank five or more cups a day had an increased incidence of angina pectoris and of myocardial infarction. Yet those who died either drank no coffee or consumed five or more cups a day.

There has long been a general feeling, without solid scientific basis, that "stress" has something to do with development of atherosclerosis. If there is anything in this concept, logically, agents which act as stimulants might be properly considered as offenders in the etiology of arterial disease. The stimulants consumed in greatest quantity throughout the world are largely those derived from xanthines: coffee, tea and beverages made from extracts of kola nuts. Coffee and the kola nut contain caffeine; tea contains caffeine and theophylline.

Certain physiologic effects of the xanthines are well known. They stimulate the myocardium to a variable extent and may increase the cardiac output. A somewhat unpredictable effect on heart rate is seen, and there is a peripheral as well as a central action on blood vessels which may result in either vasoconstriction or vasodilatation. In the cerebrovascular system, theophylline actually decreases blood flow by increasing cerebrovascular resistance. The effect of xanthines on the coronary circulation has been the subject of a great deal of study. It seems that some increase of coronary blood flow occurs, but there is also a concomitant increase in myocardial work. A variable effect on blood pressure is noted.

Recent investigation into the effect of these stimulants on blood lipids is of interest. Beller, Kornbaum and [illegible] reported a rise in plasma free fatty acids in [illegible] healthy subjects after the ingestion of coffee. Little and [illegible] found a significant correlation between coffee drinking and [illegible] and lipoprotein concentrations in men with coronary heart disease but not in healthy control subjects. There seemed to be a negative correlation with tea drinking. The authors suggested that coffee contained a substance which raised serum lipids in susceptible individuals, making them liable to coronary heart disease. On the other hand, Heyden and [illegible] [illegible]

effect of caffeine on blood cholesterol levels and on cholesterol plaques of the aorta in rabbits and concluded that cholesterol levels were actually higher and cholesterol deposits more pronounced in the control rabbits than in the animals fed supplementary caffeine.

The relation of xanthines, particularly caffeine, to carbohydrate metabolism has also been under scrutiny. Anderson, Hollifield and Owen[4] studied the uptake of glucose in isolated fat pads of rats and observed that caffeine significantly reduced glucose uptake. It has also been reported that coffee lowers glucose tolerance in maturity-onset diabetes, but this conclusion has been challenged.[5] It would appear that further study is needed to confirm or refute these observations.

Yudkin has been particularly interested in the relation of coffee and tea consumption to coronary heart disease and the possible relation of any such effect to sucrose consumption. Recently Akinyanju and Yudkin[6] reported a study of rats in which addition of coffee to the control diet significantly increased serum cholesterol and triglycerides, chiefly when sugar was present in the diet but also to a lesser extent when cornstarch was present. This effect was eliminated when decaffeinated coffee was substituted for ordinary coffee. Interestingly, tea produced a decrease in cholesterol and triglycerides. After reviewing all the evidence, Yudkin expressed the belief that it is not the caffeine in coffee and tea but sucrose which is the culprit and has the effect of increasing blood lipids. He considers this increase of blood lipids in turn to be responsible for the increased incidence of coronary heart disease reported in human beings.

DeYoung, Hotovec and Romero[7] recently reported studies of rabbits fed an atherogenic diet with or without tea supplementation. They noted lower lipoprotein levels and lesser atherosclerosis in the rabbits given tea. Their data also suggested that theophylline as a supplement might be less effective than tea itself in lowering lipoprotein levels.

Studies of the role of stimulants in heart disease in humans are contradictory. Kasanen and Forsström[8] studied the eating and smoking habits of 100 hospitalized patients 60 years of age or less who had myocardial infarction and of 100 hospitalized patients of comparable age who did not have coronary disease. The average amount of coffee consumed per day was 7.3 cups in the infarction group, compared with 4.9 in the control group. These workers expressed the belief that there was a correlation of "liberal consumption of coffee" with the cigarette habit. Walker and Gregoratos[9] in a study of 100 male military personnel 40 years of age or less at the time of myocardial infarction and of an age-matched military control group

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found that 59 percent of the infarct group consumed more than four cups of coffee a day, compared with 51 percent of the control group (not a significant difference).

In the Framingham heart study,[10] a 12 year observation of 5,127 men and women 30 to 62 years of age failed to show a significant association between coronary heart disease and coffee consumption in persons of either sex. However, the data indicate an increased coronary morbidity in persons of both sexes with increased coffee consumption, although this was not within a statistically significant range at the time of the latest report. The men and women who consumed no coffee had a morbidity ratio of 89, compared with a morbidity ratio of 129 for those consuming five or more cups of coffee a day. An earlier report[11] from the same group had failed to uncover an association between hypertension and the coffee habit.

Brown[12] investigated the records of 53[illegible] persons presumed to have died of coronary heart disease in Cheshire, England, and of 642 persons whose deaths were attributed to various other causes. Information about living habits was obtained from relatives. A tea intake of more than 10 cups a day was recorded in 14.2 percent of 409 of the coronary cases and in 11.2 percent of 483 cases in the control group. Brown also found that 28.5 percent of the coronary subjects had consumed one to five cups of coffee a day, compared with 45.1 percent of the control group. Thus it appeared that the coronary group consumed slightly more tea and somewhat less coffee than the control group.

Over the past eight years we have observed prospectively a population group of more than 1700 men 40 to 55 years old employed

Table 1. Coffee Intake and Coronary Disease*

Cups of Coffee Consumed per Month	Subjects	Angina		Myocardial Infarction†		Deaths From Coronary Disease	
		Cases	Prevalence Rate	Cases	Prevalence Rate	Number	Prevalence Rate
None	136	7	0.05	4	0.03	8	0.06
1-49	239	9	0.04	7	0.03	3	0.01
50-99	553	16	0.03	13	0.02	4	0.01
100-149	463	25	0.05	12	0.03	[illegible]	[illegible]
150 or more	327	23	0.07	17	0.05	5	0.02
	1,718	80		53		24	

*Data obtained in an eight year study of a population group of men 40 to 55 years [illegible] Western Electric Company.

†With survival.

at the Hawthorne Works of Western Electric Company in Chicago. The coffee intake and the incidence of coronary disease among this group are given in table I. It appeared that there was an increasing incidence of angina pectoris and of myocardial infarction with survival among the men consuming five or more cups of coffee a day. The coffee consumption of the men who died is interesting and puzzling. The death rate was highest among those who either took no coffee or consumed five or more cups a day. We did [illegible] evidence that the serum cholesterol [illegible] significantly higher in the group [illegible] higher coffee consumption, although it was slightly higher in these men than in those with the lowest or no coffee consumption. [illegible] with Yudkin and with Kasanen and Forsström that there were associations with sucrose intake and with the cigarette habit. It did not appear, however, that the association of coffee drinking with an increased incidence of coronary disease could be satisfactorily explained on these bases.

Summary

Observations by a number of investigators suggest that caffeine in particular has a significant effect on blood lipids and carbohydrate metabolism. Several population studies have demonstrated an association between the coffee habit and an increased incidence of coronary disease, although in some of the studies the findings do not reach the level of statistical significance. The evidence, as it is, is too suggestive and intriguing for dismissal. Likewise it is inadequate at present to permit firm and positive statements as to the etiologic significance of any of the xanthines or other stimulants.

REFERENCES

1. BELLET, S., KERSHBAUM, A. and ASPE, J.: The effect of caffeine on free fatty acids. Arch Intern Med 116:750, 1965.
2. LITTLE, J. A., SHANOFF, H. M., CSIMA, A. and YANO, R.: Coffee and serum-lipids in coronary heart-disease. Lancet 1:732, 1966.
3. HEYDEN, S. and RÜTTNER, J. R.: Die Beeinflussung des Cholesterinspiegels durch Caffein im Tierversuch. Path Microbiol 29:291, 1966.
4. ANDERSON, J., HOLLIFIELD, G. and OWEN, J. A., JR.: Inhibitory effect of caffeine on the in vitro uptake of glucose by rat epididymal adipose tissue. Diabetologia 3:50, 1967.
5. PULIER, M. and MAYER, J.: (Letters to the editor.) Lancet 1:789, 1967.
6. AKINYANJU, P. and YUDKIN, J.: Effect of coffee and tea on serum lipids in the rat. Nature 214:426, 1967.
7. DEYOUNG, W., HOTOVEC, R. L. and ROMERO, A. G.: Tea and atherosclerosis. Nature 216:1015, 1967.
8. KASANEN, A. and FORSSTRÖM, J.: Eating and smoking habits of patients with myocardial infarction. Ann Med Intern Fenn 55:7, 1966.
9. WALKER, W. J. and GREGORATOS, G.: Myocardial infarction in young men. Amer J Cardiol 19:339, 1967.
10. KANNEL, W. B.: The Framingham Heart Study. U.S. Public Health Service Publication No. 1515. Washington, D.C., U.S. Government Printing Office, 1966.
11. DAWBER, T. R., KANNEL, W. B., KAGAN, A., DONABEDIAN, R. K., MCNAMARA, P. M. and PEARSON, G.: Environmental factors in hypertension. In STAMLER, J., STAMLER, R. and PULLMAN, T. N. (Editors): The Epidemiology of Hypertension. New York, Grune & Stratton, Inc., 1967, p 255.
12. BROWN, A.: Coronary thrombosis. An environmental study. Brit Med J 2:567, 1962.

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A Longitudinal Study of Coronary Heart Disease

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SINCE the Fall of 1957, a long-term study of coronary heart disease has been in progress at the Hawthorne Works of the Western Electric Company in Chicago under the auspices of the University of Illinois College of Medicine and Presbyterian-St. Luke's Hospital. The study was undertaken in the belief that coronary heart disease was a disease resulting from the interplay of multiple factors and that there was need to delineate these factors further. The data presented herein represent the initial compilation of data centering on this problem; a discriminate function analysis is to be undertaken next. The report which follows has been made possible through the efforts of a large group of physicians and other scientists who have volunteered their time in the project to permit the accumulation of a large body of data, a portion of which is presented below. Acknowledgment must also be made to the Western Electric Company, which has been most cooperative and helpful throughout, to its employees who have participated in the study, and to the agencies and donors partially recognized below* who have financed the survey since its inception.

Plan of Study

It was planned to study the characteristics of a male population believed to be free from clinical coronary disease through annual interviews and examinations carried on over a period of at least 5 years. The attributes of those men developing clinical coronary disease in the course of the study were to be compared to those men not so afflicted. The Hawthorne Works of the Western Electric Company was selected both because of its reasonable proximity to the Medical Center, and because of the expressed interest of its Medical Department in such a project. Over 20,000 persons were employed in the Hawthorne Works in 1957, mainly in clerical and light assembly work, although a significant group was employed at positions requiring vigorous labor. The group was composed chiefly of second- and third-generation Americans of Polish and Bohemian ancestry.

All men aged 40 to 55 who had been employed at the Works for 2 or more years (N = 5,397) were assigned an identification number by a randomizing process. Members of the randomly selected group were then invited to participate in the study—participation was entirely voluntary—by being called from the random list. In this process the medical records of the Company were inspected and those men for whom there was evidence of angina pectoris by description or of myocardial infarction confirmed by history and electrocardiographic changes were excluded, as were men in certain additional categories (deaths subsequent to preparation of the list, transfers, permanent disabilities, leaves of absence); this group numbered 127 men. Invitations to participate were sent by letter after preliminary discussion and explanation at the top management and supervisor level, and publicity through the plant bulletin and company magazine. It was planned to invite a sufficient number to provide a population of approximately 2,000 men. Of the 3,102 men asked to participate, 2,080 or 67 per cent of those invited agreed to participate and were examined the first year. Of this group,

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44 were excluded from subsequent analysis because the first examination indicated the presence of coronary heart disease. Forty-seven others were not included in the analysis because of special problems in follow-up unrelated to the cardiovascular system. The base population therefore available at the end of the first year for analysis amounted to 1,989 men. At the end of 4 years, 38 men had died and 105 men had withdrawn from the project or left the Company. Over 94 per cent of the survivors of the original group had thus remained in the study. The group labeled "non-coronary" in subsequent references will be the base population less men not followed, the new coronary cases, and in certain instances a small number of men for whom adequate data were lacking. After the first year the men were, in general, examined on an anniversary-month basis, although in many instances, this was not possible due to commitments at work, travel, or illness.

No therapeutic suggestions of any kind were given to the men participating in the study, and the study itself was labeled a Health Survey to draw attention away from the cardiovascular system.

The diagnosis of angina pectoris was made by two or more internists in each case and was based upon published criteria.[1] The diagnosis of myocardial infarction was made with use of the clinical history plus characteristic electrocardiographic findings.[1] Where difference of opinion occurred, decision as to diagnosis rested with the senior investigator, who undertook an additional interview and physical examination. The assumption of death from coronary disease was made on the basis of the most reasonable conclusion to be drawn from information obtained from the family, physicians' and hospital records, death certificates, and coroners' reports. Unfortunately, postmortem examinations were rarely made.

A family history was provided by each participant, who completed a form with the help of his family. Annual additions to the history were made by interview. The medical history and physical examination were modeled after that employed in the Framingham Project.[2] The dietary data were obtained by a 1-hour interview with a trained dietitian.[3] Supplementary information was obtained from forms completed by the wives, and by interviews with a sample of the wives. Plastic food models were employed to assist in portion estimates, as were data regarding food purchases and preparation obtained from the Company cafeteria.

Physical activity on the job was assessed by reference to a Company code, the validity of which was confirmed for work in the shops by oxygen studies on the job in a small sample of 36 men. The code included five grades of physical activity in the shops and four of environmental factors in the offices. Physical activity off the job was estimated by use of a questionnaire administered by a trained interviewer from the personnel department of the Company.

A routine 7-foot chest x-ray was taken, as were an electrocardiogram with a direct-writing instrument with standard 12 leads plus lead V_{4R}, urinalysis, blood hemoglobin level (with a colorimeter), and blood cholesterol (Abell-Kendall method). Body fatness was measured with a skin-fold caliper. Somatotyping was performed by standard measurements plus photographs in three views of each man clothed in a brief supporter, with typing by the Sheldon method.[4] Lipoprotein lipase measurements were made the third year by the technic of Pilgeram.[5]

Blood pressures were taken at the beginning of each physical examination with the participant seated; left and right arm measurements were found to be similar on statistical analysis and the left arm readings are referred to below.

Psychologic studies were also made and will be reported separately.

For the purpose of this report, the diagnosis of angina pectoris is given when a man exhibited only this manifestation of coronary disease in the absence of aortic stenosis or other obvious underlying factors. The diag-

nosis of myocardial infarction is given when myocardial infarction was adequately documented whether or not angina had been present on previous examinations (this occurred in one of 28 infarcts). The diagnosis of death from coronary disease is listed regardless of previous documentation of angina pectoris or myocardial infarction, providing adequate evidence for such listing was available. A history of sudden death was not considered adequate evidence by itself.

In the data presented below, differences between the groups are investigated with respect to data obtained from the first examination unless otherwise stated.

Results

New Coronary Cases

After 4 years and 5 months of the study, 88 men had been classified as having developed clinical coronary heart disease as follows: angina pectoris, 47 men; myocardial infarction, 28 men; deaths from coronary disease, 13 men. Approximately one new case per 100 men per year was thus diagnosed. These coronary men had a mean age 6 months older than the men in the noncoronary group, with a distribution curve skewed somewhat to the right.

The group under study was probably not more or less coronary prone than the men who were invited but did not accept or respond. This is shown by the fact that at the end of 4 years and 5 months, 21 of the 1,022 who declined or did not respond had died from all causes and 45 of the 2,080 who had accepted had died from all causes. The mortality percentages are 2.1 and 2.2 per cent, respectively. The total mortality for the 2,168 men in the group of 5,397 whose number never came up for consideration was 3.3 per cent, which compares with a total mortality of 3.1 per cent for the 3,102 invitees plus the 127 men referred to previously who were considered but not called.

Table 1

History of "Noncardiac" Chest Discomfort

	Noncoronary cases (1,734)	Coronary cases (87)
Yes	16%	31%
No	84%	69%

Data relating to history of "noncardiac" chest discomfort as obtained at the time of the initial examination and its relation to subsequent recognition of clinical coronary heart disease.

Historical Data

Family History

There was no significant difference between the coronary and noncoronary groups when analyzed for the historical evidence of both parents dying over 70 years of age, over 65 years of age, or dying over 60 years of age. The ages of living parents were adjusted to expected age at death according to the American Experience Mortality Table. There was no significant difference between the average age of death of the mother of these two groups, but there was with the age of the father (the age of the fathers in the noncoronary group exceeded that of the coronary group by 3.4 years, on the average). Information on the grandparents' age at death did not appear to be a factor associated with the incidence of coronary disease.

History of Chest Discomfort

It was of considerable interest to follow the men who at the time of the first examination gave a history of some type of chest discomfort that was not considered adequate for a diagnosis of angina pectoris. Table 1 shows a significantly higher development of clinical coronary disease in those men who gave such a history, as contrasted with those who did not give such a complaint ($p < 0.001$).

History of Cough and Shortness of Breath

A chronic cough was stated to be present by 25 per cent of the men in the coronary group (88 cases) as contrasted with 12 per cent of the noncoronary men (1,778 cases). Likewise, 18 per cent of the men in the coronary group indicated shortness of breath on effort as compared with 11 per cent of the men in the noncoronary category. Both of these differences are significant ($p < 0.001$ for the former and < 0.025 for the latter).

Table 2

Somatotype Distribution in Noncoronary and Coronary Groups

Category	Noncoronary cases (1790)	Coronary cases (87)
	Obs./Exp.	Obs./Exp.
Endo. dominant	253/259	19/13
Meso. dominant (endo. > ecto.)	742/743	37/36
Meso. dominant (endo. = ecto.)	156/153	4/7
Meso. dominant (endo. < ecto.)	124/120	2/6
Endo. = Meso.	217/221	15/11
Meso. = Ecto.	101/99	3/5
Ecto. dominant	197/195	7/10

Somatotype distribution in the noncoronary and coronary groups, listing observed and expected numbers. In the mesomorphic dominant category are listed the secondary dominant or equal components.

History of Peptic Ulcer and Gallbladder Disease

Ten per cent of 1,794 noncoronary men gave a history the first year of peptic ulcer as compared with 18 per cent of the 88 coronary cases: this difference is significant ($p < 0.025$). There was no significant relationship with a history of gallbladder disease.

Weight Gain

The pattern of weight gain and loss from age 20 to age 40 was not appreciably different in the two groups.

Physical Characteristics and Observations

Height and Weight

The mean height and weight for the noncoronary population (1,776 men) were 68.7 inches and 171.1 pounds, and for the coronary group (88 men) 68.4 inches and 172.0 pounds. These differences are not statistically significant. The correlation coefficients for height versus weight in the two groups also were not significantly different.

Skin Fatness

A significant difference in skin fold thickness for the same two groups was demonstrated: the mean triceps skin fold thickness measured 1.39 cm. for the 1,776 noncoronary men and 1.52 cm. for the coronary group, and the mean scapular thicknesses were 1.81 cm. and 1.98 cm., respectively. The difference between the two groups is significant at the 0.025 level for the triceps measurement and at the 0.01 level for the scapular thickness.

Somatotype Data

Somatotype of individuals was scored by the Sheldon technic. The subjects fell naturally into three groups: (1) endomorphic dominance, i.e., the endomorphic component exceeded the other two components, (2) ectomorphic dominance, and (3) all those whose mesomorphic component was at least as high as either of the other components. The last category is by far the largest. It was decided that a closer scrutiny of the mesomorphic dominant group was desirable. Accordingly, the mesomorphic group was further partitioned into groups defined by the comparative sizes of the other components. The groups actually considered are clearly defined in table 2.

It has been reported that mesomorphic dominance in body build is positively related to the presence of clinical coronary disease.[6] Our material does not support such a conclusion, and instead suggests that endomorphic dominance may be important, in that there is an excess of coronary cases in the group characterized by endomorphic dominance ($p < 0.1$). A larger series will be needed to confirm this latter point.

Pulse Rate

The mean pulse rate of the coronary group (80.7) was not significantly higher than that of the control group (78.5). The distribution of pulse rate in each group was markedly different, however, with a p value of 0.025

Table 3

Pulse Rates in Noncoronary and Coronary Group

Rate	Noncoronary cases (1,466)	Coronary cases (88)
< 69	16%	18%
70-79	38%	30%
80-89	31%	25%
90-99	11%	16%
> 100	5%	11%

Table 4

Mean Blood Pressure in Noncoronary and Coronary Groups (Left Arm Readings)

Noncoronary cases (1,788)	Coronary cases (87)
135	146
87	92

(table 3). In particular, the coronary group appeared to have a higher percentage of extreme cases.

Blood Pressure

Table 4 lists the data relating to blood pressure recordings in the coronary and the noncoronary categories. As reported by others, a positive relationship is apparent between elevations of the systolic level and the development of coronary disease ($p < 0.001$) and also with the diastolic level ($p < 0.05$). The mean systolic blood pressure of the coronary cases was 146 as compared with 135 for the noncoronary population, and comparable figures for the diastolic level were 92 and 87. Isolated systolic blood pressure elevation by itself was uncommon. The mean diastolic level for the angina group was 92, for the infarct group 90, and for the men who died from coronary disease 94. Sixteen per cent of the noncoronary group had a diastolic level of 100 or over; this rose to 28 per cent for the angina group, 29 per cent for the infarct group, and 33 per cent for those who died from coronary disease. A discussion of the blood pressure data in relation to the family history has been presented elsewhere.[7] It is well to remember that in any such population study, fluctuations of the blood pressure levels with the season of the year occur and that a survey limited to a single season may give different results from one in which the data were obtained at another time of year (fig. 1).

Ophthalmologic Observations

No relation between the presence of arcus senilis and coronary heart disease was noted. There were not enough examples of xanthelasma to permit statistical analysis. It was somewhat surprising to find that a definite statistical significance was present between the observation of arteriovenous nicking as noted the first year and the subsequent development of coronary disease. In 1,793 noncoronary men and 86 coronary cases in whom data were available, the percentage having this finding was 4 per cent and 13 per cent respectively, a difference which is significant at the 0.005 level.

Presence of "Tension"

In view of the preoccupation of some investigators with impressions of "tension" or tenseness in coronary or supposed coronary-prone individuals, it is worth while to record that the internists making these examinations were asked to check at the end of the examination (which occupied approximately 45 minutes) whether they considered the man examined to be "relaxed" or "tense." No significant difference was seen but there was a slight excess of men classified as "relaxed"

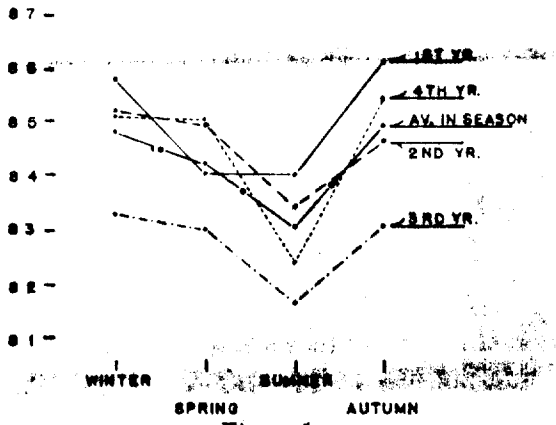

Figure 1

Pattern of seasonal blood pressure change (diastolic pressure only) as observed over 4 years. The systolic pattern is similar.

who subsequently developed clinical coronary disease.

Laboratory Observations

Hemoglobin Levels

No significant difference was shown among the noncoronary, angina, and infarct groups in mean hemoglobin levels although the trend was in the direction noted by others. The mean hemoglobin level for the noncoronary men was 14.8 Gm. per cent, for the angina cases 15.0 Gm. per cent, and for the infarct cases 15.2 Gm. per cent.

Cholesterol Levels

As anticipated, a definite relationship was demonstrated between higher blood cholesterol levels as found the first year, and subsequent incidence of clinical coronary heart disease (table 5). This relationship is highly significant ($p < 0.01$). The mean cholesterol level for the noncoronary population was quite high, 247 mg. per cent, and for the coronary groups was 272 mg. per cent. Despite this mean difference, almost 30 per cent of the coronary cases had blood cholesterol levels below 240 mg. per cent.

A second and perhaps more important observation was the demonstration of a significant difference in behavior of the coronary subgroups. A comparison of cholesterol levels in the noncoronary cases with the levels of 17 angina cases, the 28 infarct cases and 13 men who died of coronary disease, is indicated in table 6. The stepwise progression of the mean cholesterol levels is clearly seen, from the lowest levels in the men as yet not identified as having coronary disease to the highest level in those who have died from coronary disease.

Table 5

Cholesterol Levels in Noncoronary and Coronary Cases

Mg.	Noncoronary cases (1794)	Coronary cases (88)
100–204	20%	7%
205–239	29%	22%
240–256	15%	15%
257–274	11%	17%
275–309	14%	22%
310	12%	18%

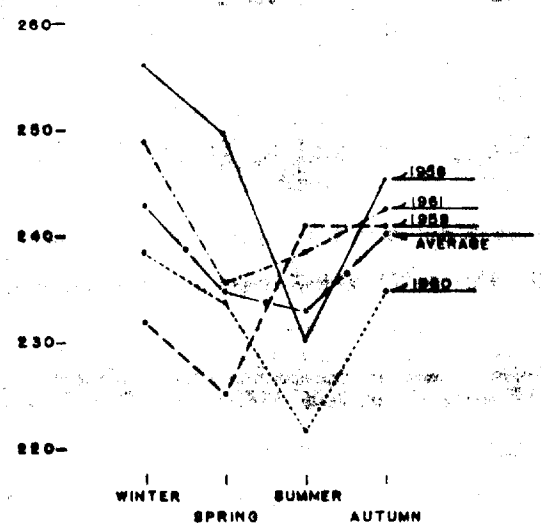

Figure 2

Seasonal variation in serum cholesterol levels as observed over 4 years. It will be noted that only one season is inconsistent with the trend (Summer 1959). The reason for this is unknown.

A third observation in the cholesterol determinations supports the work of Thomas et al.[8] These authors found a seasonal variation in cholesterol levels in a small group of prisoners who were studied throughout the year. As will be seen in figure 2, our data show a consistent seasonal variation, the highest levels being encountered in the winter with the lowest in the spring or summer. In this study, cholesterol determinations were not made on each man for each season; rather, the means for each season were determined with the data procured from annual examinations. The men were often but not invariably examined in the same season each year. That the variation is not identical with the season variation in blood pressure recordings can be seen by comparison with figure 1. Our dietitians have also analyzed seasonal dietary changes and find that in this population, they are apparently insignificant although more beer is consumed in the months with higher temperatures and somewhat more fruit is included in the diet. There is no change in the

Table 6

Cholesterol Levels in Noncoronary and Coronary Subgroups

	Noncoronary cases (1794)	Angina (47)	Infarct (28)	Deceased (13)
Mean	247.3	263.6	275.2	284.5
< 256 mg. %	63%	48%	43%	23%
> 256 mg. %	37%	52%	57%	77%

hours of work or type of work related to the seasons although we have determined that men are more physically active in the summer than the winter. The magnitude of the variations present is considerable and important in any study in which attempts to manipulate the blood cholesterol levels are made.

Lipoprotein Lipase Measurements

During the third year and during the first half of the fourth year, lipoprotein lipase determinations were made on the whole group. Lipoprotein lipase cofactor levels were also made. These studies were carried out through the courtesy of Dr. John Olwin of the Coagulation Laboratory of Presbyterian-St. Luke's Hospital. There was no evidence of a relationship between the presence of clinical coronary disease and levels of either lipoprotein lipase or lipoprotein lipase cofactor.

Blood Sugar Determinations

During the third year of the project, each man was asked to drink a solution containing 100 Gm. of glucose, and a blood sample for glucose determination was drawn approximately 2 hours later. The mean blood-glucose levels showed no significant differences between the noncoronary and coronary groups (mean noncoronary blood-glucose level 106.7 mg. per cent, mean coronary level 106.8 mg. per cent). Of interest was a significant heterogeneous variation within each of the four coronary groups. The angina group was the most variable, followed by the noncoronary and infarct groups, respectively. The deceased group appeared to be the least variable, but the estimated standard deviation is based on only five observations. The difference in variability between the angina and infarct group was particularly striking.

Electrocardiograph Findings

A limited survey of the electrocardiographic observations has shown as yet no significant association between the presence of ventricular premature beats or the finding of a complete left or right bundle-branch block with the development of clinical coronary disease. The numbers in these categories are as yet small. However, there was an association found between all types of ST-segment or T-wave abnormalities (in the absence of a complete bundle-branch block or QRS abnormalities consistent with *myocardial* infarction). Thus 10 (11 per cent) of the 88 electrocardiograms of the men subsequently identified as coronary cases showed these changes on the first electrocardiographic tracing, whereas only 49 (2.7 per cent) of the 1,757 electrocardiograms in the as yet noncoronary group revealed this type of abnormality. This is highly significant ($p < 0.005$).

Environmental Factors

Place in the Company

There was no indication that current type of work in the Company was a factor predisposing to the development of coronary disease (table 7). A study of the prior work history likewise failed to demonstrate any significant differences.

Physical Activity off the Job

Physical activity off the job was estimated from the answers to series of questions posed to each man by a trained interviewer. These questions included reference to blocks walked to and from work, flights of stairs climbed, hours devoted to household chores and yard work, and past and present participation in sports. No striking differences were encountered between the coronary and noncoronary

groups, although, in general, men in the former category seemed to spend more time in household and yard work and fewer hours a week in sports than did men in the latter category. Thus 38.6 per cent of the coronary men denied participation in bowling, golf, baseball, hunting, or fishing as contrasted with 23.4 per cent of noncoronary men, and when the coronary men did engage in these activities, they tended to do so for shorter periods.

Tobacco

No difference was found between the groups relative to the habit of cigar smoking, or to the habit of pipe smoking. A highly significant relation to the use of cigarettes was apparent. The data secured in answer to the question asked of the participants to indicate the number of cigarettes smoked most of their adult life are seen in table 8. The association of cigarette smoking most of adult life with the subsequent development of clinical coronary disease is significant, with a p value of 0.005. It is also significant in regard to current smoking habits, also with a p value of 0.005. Further, it was noted that there

Table 7

Job Classification

		Noncoronary cases (1797)	Coronary cases (88)
Shop occupations physical demand code	1	.3%	
	2	38.0%	29.5%
	3	45.6%	47.0%
	4	4.0%	5.7%
	5	.2%	
Office occupations work condition code	1	32.2%	39.8%
	2	8.2%	6.8%
	3	1.4%	1.1%
	4	.2%	

Job classification of noncoronary and coronary cases. Code 1 in the shop represents the least physical effort; code 5, the most strenuous and sustained effort. Code 1 in the office represents the most ideal environmental conditions in terms of noise, temperature change, etc.; code 4, the most disturbing type of environment.

Table 8

Cigarettes Smoked "Most of Adult Life"

Number of cigarettes per day	Noncoronary cases (1786)	Coronary cases (87)
Nonsmoker	33%	23%
1/4 Pack (1-7)	7%	2%
1/2 Pack (8-12)	11%	9%
3/4 Pack (13-17)	12%	6%
1 Pack (18-22)	30%	47%
1-1/4 Pack (23-27)	[illegible]	[illegible]
1-1/2 Pack (28 +)	6%	9%

was a relation between the number of cigarettes smoked and the manifestation of coronary disease identified (table 9), which relation was significant at the 0.005 level. The stepwise trend analogous to that observed with cholesterol levels is to be noted.

Diet

The assessment of diet[3] was done by interview as indicated above. It is recognized that such a method yields at best only an approximation of food intake. In a population of this size, metabolic balance studies were clearly out of the question. Attempts at validation of the interview technic were done as mentioned above; these showed an excellent correlation when a sample of the men (20) were interviewed by two dietitians on different days. A comparison of the interview method with records kept by the men (50 the first year, 364 the third year) in a specially prepared 7-day food-intake diary revealed that the interview approach (which embodied a 1-week meal pattern plus a 28-day recall) gave higher levels for 21 nutrients than did the diary kept by the men. It appears that the interview tends to exaggerate to some extent the quantities of food taken in, while the diary underestimated quantities. The men often conceded that they were embarrassed to list in some cases all that they really had consumed. Because of the extensive experience of our dietitians with these men, the information obtained from the wives and other sources to supplement the data, and the similarity of the information obtained on successive years (being described in greater detail

Table 9

Cigarette Data in Noncoronary and Coronary Subgroup

	Cigarettes adult life				Cigarettes now			
	Noncoronary cases (1786)	Angina (47)	Infarct (27)	Deceased (13)	Noncoronary cases (1789)	Angina (47)	Infarct (28)	Deceased (13)
Nonsmokers and smokers of 1/2 pack per day or less	51%	40%	26%	31%	58%	51%	36%	31%
Smokers of more than 1/2 pack per day	49%	60%	74%	69%	42%	49%	64%	69%

elsewhere), we believe the dietary data to be a reasonable approximation of the truth.

Table 10 lists the dietary information for the coronary and noncoronary groups. No significant differences are apparent for these nutrients.

In an attempt to isolate the high- and low-fat groups from the point of view of risk, the 296 men comprising the top 15 per cent of the whole population in terms of calories from fat were contrasted with 296 men representing the bottom 15 per cent in terms of fat intake. It will be seen from table 10 that only 20 per cent of the fat calories came from vegetable fat, so that this is in essence a comparison of high and low animal fat groups. Table 11 gives the important findings in these two populations. Worthy of comment is the fact that of the 88 coronary cases, 11 have appeared in the high fat intake group and 16 in the low-fat group.*

Table 10

Daily Average for Nutrients

	Noncoronary cases (1797)	Coronary cases (88)
Total calories	3174	3082
Animal protein (Gm.)	90	90
Vegetable protein (Gm.)	28	26
Animal fat (Gm.)	118	116
Vegetable fat (Gm.)	34	32
Carbohydrate (Gm.)	304	299
Calcium (Gm.)	1.08	1.10
Phosphorus (Gm.)	2.33	2.16
Iron (Mg.)	17.2	16.6
Cholesterol (Mg.)	757	721
Total sat. fatty acids (Gm.)	59	59
Total unsat. fatty acids (Gm.)	83	80
Linoleic acid (Gm.)	12.28	11.57
Linolenic acid (Gm.)	.69	.66
Arachidonic acid (Gm.)	.98	.97
Vitamin "A" (I.U.)	12,470	11,750
Thiamine (Mg.)	1.66	1.55
Riboflavin (Mg.)	2.52	2.51
Niacin (Mg.)	22.8	22.2
Vitamin "C" (I.U.)	105	101
Vitamin "D" (I.U.)	198	196

There was no demonstrable relation between responses to questions regarding salt intake in the coronary and the noncoronary groups. The second year, the men were, in addition, asked to taste three samples of soup containing no salt, a moderate amount of salt, and a heavy amount of salt to indicate their preference as to flavor. Here, too, no positive relationship could be found.

A positive relation was encountered, however, between coffee intake and coronary disease. Because of subsequent discovery of variations in portion size for coffee intake during some of the first interviews, only the data for a total of 1,108 of the men seen consecutively during the last 9 months of the first year are listed in table 12. For this group, a significant correlation between the use of coffee and the later development of coronary disease is seen ($p < 0.025$).

*We have attempted to assess the constancy of diet in these two groups. In the third year of the project, a complete diet interview was performed in a sample composed of 60 men from each category. At this time, the sample group initially characterized as having a high fat intake obtained 45 per cent of its calories from fat, and the sample group from the original low fat category obtained 39 per cent of its calories from fat. The difference in the fat intake of the two groups has thus lessened, a result not unexpected in view of the phenomenon of regression toward the mean upon repeated testing.

Table 11

Comparison of Groups with High and Low Fat Intake

	High fat (296 cases)	Low fat (296 cases)
Median % calories from fat	49.3	36.1
Average cholesterol (Mg. %)	253.8	246.8
Average height (inches)	69.0	68.4
Average body weight (lb.)	168.9	175.0
Average triceps skin fold (cm.)	1.1	1.1
Average scapular skin fold (cm.)	1.8	1.9
Average blood pressure (left arm)	133/86	130/90
Shop work	171 Cases-median grade 2.3	176 Cases-median grade 2.4
Office work	121 Cases-median grade 1.2	120 Cases-median grade 1.2
New coronary cases	14	16

No positive association was found between the development of coronary disease and the intake of alcoholic beverages.

Discussion

The data presented clearly cannot have too broad an application. The population under survey was limited to men in an age group known to be susceptible to coronary disease. Further, the population is an industrial one and in background relatively homogeneous and stable. Also, as yet, only a moderate number of individuals have been identified as having clinical coronary disease. As we have watched the data unfold, however, there has been a remarkable consistency to it, and a trend once revealed has shown greater and greater statistical significance as new cases have been added. We have waited to this point before presenting the material available in order to try to have a body of data reasonably suitable for analysis. As one fragments the coronary group into subgroups, the numbers of course become smaller and conclusions are tenuous.

The picture that has unfolded is clearly that of a multifactorial disease. Positive relationships have been established between coronary disease and the presence of elevation of systolic and diastolic blood pressure, elevations of the blood cholesterol levels, and heavy use of cigarettes as reported by others. The association with abnormalities in the electrocardiogram was also not unexpected. Of particular importance is the link between a history of any type of chest discomfort and the later recognition of coronary disease. This finding confirms what many clinicians have observed. It should be commented that it is probably more difficult to elicit a history of classical symptoms of disease in men who come for this type of screening examination as compared with those who present themselves at the office of a physician proffering a complaint. This also further points to the vagaries of angina pectoris in its manifestations. Agreement with the criteria employed in the diagnosis of angina pectoris in this study was evidenced by a review of the first 30 of the angina cases by a team of three physicians (one from Chicago, one from Alabama, and one from England).[9] The importance of the association with a prior history of peptic ulcer is uncertain, but may reflect certain of the problems to be discussed in the companion paper on the psychologic studies completed on the group.

Table 12

Intake of Coffee

Cups/month	Noncoronary cases (1108)	Coronary cases (14)
0–49	17%	16%
50–99	36%	28%
100–149	26%	15%
150–199	15%	22%
200	7%	19%

It was particularly interesting to us to encounter the stepwise association of the blood cholesterol level with the coronary subgroups. To those who decry the significance of blood cholesterol findings in population groups, this should be food for thought. This evidence helps to buttress the concept that the patient with angina pectoris is not identical with the patient who develops a myocardial infarct, or even who dies from the effects of the underlying disorder. The limited extent to which this is meaningful in the individual patient is however obvious.

We regret our failure to find any relation between the clearing factor, lipoprotein lipase, and this type of vascular disease. Certainly, this is a field that needs more extensive plowing and cultivation.

Finally, our dietary data with all the limitations of the methods employed to obtain them appear worthy of presentation and valuable as a basis for comparison with other groups. It is true that our population does not include a low-fat group comparable to populations found in certain other parts of the world. Perhaps, more important, the figures presented do show the dietary profile of a population group living under free conditions and exposed to a combination of environmental influences not unlike those prevalent in most other parts of the United States. The findings noted contrast with the step-wise association observed with certain other factors and would seem to inject a healthy note of caution at least toward attempts to alter the American diet within the modest range of fat intake we have described.

Summary

Certain of the data accumulated over the course of more than 4 years in a prospective study of coronary heart disease being carried on in an industrial population have been presented.

In a base population of 1,989 men, 88 cases of coronary heart disease have developed: angina pectoris, 47 men; myocardial infarction, 28 men; death from coronary disease, 13 men. This approximates one case per 100 men per year.

The development of clinical coronary heart disease has shown an association with early age of death of father, history of "nonCardiac" chest discomfort, history of chronic cough, history of shortness of breath, history of peptic ulcer, presence of increased skin fold thickness, elevated blood pressure, AV nicking in the fundi, elevated blood cholesterol, ST and T abnormalities in the electrocardiogram, and use of cigarettes and coffee.

No relation was encountered between body weight, mean blood sugar levels, lipoprotein lipase levels, or diet (other than coffee), and the development of coronary heart disease. Similarly, there was no association with job type and no certain relation to physical activity off the job.

Acknowledgment

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Appendix

In relation to the somatotype material, reference no. 4 quoted, page 5, gives the following description of the three components used. "Endomorphy means relative predominance of soft roundness throughout the various regions of the body." "Mesomorphy means relative predominance of muscle, bone, and connective tissue." "Ectomorphy means relative predominance of linearity and fragility." The individuals were rated on a scale of one to seven for each of these components.

In relation to job classification, it may be helpful to indicate some of the definitions employed in the Company physical demand table for work in the shop. For the purpose of job evaluation, "continuous" means 50 per cent or more of the time, "frequent" 10 to 50 per cent of the time, "occasional" 2 to 10 per cent of the time. Thus, code 2 involves (a) work that requires the continuous lifting of

increasing severity of degree of atherosclerosis with increasing elevation of the blood cholesterol levels has been noted experimentally."

material weighing over 1 pound and up to 5 pounds inclusive, or frequent lifting of material weighing over 5 pounds and up to 25 pounds inclusive, or occasional lifting of material weighing over 25 pounds and up to 60 pounds inclusive or equivalent exertion pushing or pulling; or (b) work that requires the employee to stand on one work position or walk continuously or work involving continuous use of arms raised in an unsupported position, lifting or holding material weighing up to 1 pound inclusive. Code 4 involves (a) work that requires the continuous lifting of material weighing over 25 pounds and up to 60 pounds or equivalent exertion pulling or pushing; or (b) work that involves continuous strain due to strenuous work positions.

The work-condition code in the office occupations refers to requirements or conditions incidental to the occupation or to surroundings that result in the tiring or discomfort of the individual. These include abnormal visual demand, physical effort or endurance, distinctly objectional surroundings involving heat, cold, dirt, noise, fumes, or hazards. Thus, the first-degree code in the office is essentially normal office surroundings with minimum tiring effect, such as would be necessarily present in any job. The second-degree code includes some one individual objectionable surrounding or physical or visual demand existing to a degree distinctly beyond that encountered in normal salaried occupations. The third code is a combination of two or more objectionable conditions, each of which qualifies an occupation for no. 2 or some one individual, [illegible] objectionable, surrounding condition.

[illegible] those statistically minded, the following com[illegible] may be made. The chi-square test of sig[illegible] was employed in determining the p values for history of chest discomfort, history of cough [illegible] of breath, history of peptic ulcer, [illegible] data, distribution of pulse rate, finding [illegible] nicking, relationship of cholesterol [illegible] coronary disease, the electrocardiographic [illegible], and for the cigarette and coffee data. On [illegible] other hand, t tests were appropriate for the height and weight data, body fatness measurements, mean pulse rates, mean blood pressure levels, and mean cholesterol levels. Analysis of variance was employed for the cholesterol levels in the coronary subgroups, and Welch's approximation was used for the mean blood glucose levels.

References

1. Epidemiology of Cardiovascular Diseases: Methodology. Report of Princeton Conference. Am. J. Pub. Health, Suppl. 10, 50: 1, 1960.
2. Dawber, T. R., Meadors, G. F., and Moore, F. E.: Epidemiological approaches to heart disease: The Framingham Study. Am. J. Pub. Health 41: 279, 1951.
3. Paul, O., and Macmillan, A.: Nutritional habits of midwestern workingmen. Illinois M. J. 120: 273, 1961.
4. Sheldon, W. H., Stevens, S. S., and Tucker, W. B.: The Varieties of Human Physique. New York and London, Harper, 1940.
5. Pilgeram, L. O.: Deficiencies in the lipoprotein lipase system in atherosclerosis. J. Gerontol. 13: 32, 1958.
6. Gertler, M. M., and White, P. D.: Coronary Heart Disease in Young Adults: A Multidisciplinary Study. Cambridge, Harvard University Press, 1954.
7. Ostfeld, A., and Paul, O.: Some observations on the inheritance of hypertension. Lancet. In press.
8. Thomas, C. B., Holljes, H. W. D., and Eisenberg, F. F.: Observations on seasonal variations in total serum cholesterol level among healthy young prisoners. Ann. Int. Med. 54: 413, 1961.
9. Rose, G. A.: The diagnosis of ischemic heart pain and intermittent claudication in field surveys. Bull. World Health Organ. In press.
10. Hass, G. M.: Experimental inquiry into pathogenesis of human atheroarteriosclerosis. Geriatrics 16: 581, 1961.

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Caffeine Toxicity in Starved Rats[1]

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Reduced food intake and weight loss are common occurrences in animal chronic toxicity studies. While these factors are related to drug effect, they may themselves influence drug response (Friedman, 1966).

Reduced food intake can complicate the experiment in several ways. Oral administration of a drug on an empty stomach usually increases its toxicity. The acute oral LD_{50} of caffeine is 192 ± 18 mg/kg in normal starved rats (Boyd, 1959) and 264 ± 10 mg/kg in normal, unstarved rats (Boyd *et al.*, 1965). Starvation can also affect the metabolism of certain drugs by depressing the activity of liver microsomal enzymes (Dixon *et al.*, 1960).

Underweight and poor health are generally considered to augment susceptibility to drug toxicity. Salant and Rieger (1912), for example, found that animals "with pathological conditions" were more sensitive to caffeine toxicity. In our laboratory, animals with a low body weight and food intake during the control day before drug administration often have been found more susceptible to the toxic effects of caffeine.

In the present experiments, partial starvation was induced by limiting the food intake, and its effect on caffeine toxicity was studied in albino rats.

METHODS

The animals were adult female albino rats of a Wistar strain which had been bred in the animal quarters of this department. They were fed a pulverized laboratory chow,[2] housed separately in metabolism cages, and transferred daily to clean ones. Water was supplied ad libitum. Food was offered ad libitum in a nonspilling feeding device or as a weighed amount in a small cup. Daily clinical measurements included body weight, food intake when food was offered ad libitum, water intake, urinary volume, and the pH, protein and glucose content of a 24-hour urinary specimen.[3]

Caffeine was used as caffeine alkaloid monohydrate USP. A dose of 185 mg/kg was administered daily by intragastric tube, dissolved in 20 ml of distilled water. At this dosage a 50% mortality in 17 days was predicted from the data of Boyd *et al.* (1965).

Treatments of the various groups are summarized in Table 1. The body weight of animals in groups 1 and 2 at the end of 2 weeks had reached (as predicted) the

[1] Presented in part at the Seventh Annual Meeting of the Canadian Federation of Biological Societies, Halifax, Nova Scotia, June 10-12, 1964.

[2] Purina Laboratory Chow Checkers, Ralston Purina Company, Limited, Woodstock, Ontario, Canada.

[3] The last three measurements were determined with Ames Combistix®, Ames Company of Canada, Limited, Toronto, Ontario, Canada.

same level as the initial body weights in groups 3-8. Eight grams of chow daily was taken as the upper limit of food reduction, because a higher daily allowance was not always eaten.

Caffeine administration was started after one control day of reduced food intake without caffeine. To avoid administration on an "empty stomach," caffeine was given 30-60 minutes after the offering of food.

TABLE 1

GROUPS, NUMBER PER GROUP, INITIAL BODY WEIGHT, AND TREATMENT OF RATS GIVEN REDUCED FOOD INTAKE AND CAFFEINE, AND THEIR MORTALITY RATES

Group number	Number of animals	Initial body weight[a] (g)	Treatment	Food intake[b] (%)	Mortality %	Mortality: Time to 50% mortality (days)
1	10	185 ± 10	Chow ad libitum + distilled water	100 (14.1 ± 0.7)[d]	0	—
2	9	177 ± 13	Chow ad libitum + caffeine[c]	104 (14.6 ± 2.7)[d]	0	—
3	8	198 ± 12	8 g chow daily + distilled water	57	0	—
4	10	191 ± 18	8 g chow daily + caffeine[c]	57	0	—
5	6	195 ± 10	6 g chow daily + distilled water	43	0	—
6	8	198 ± 14	6 g chow daily + caffeine[c]	43	88	12
7	9	194 ± 14	4 g chow daily + distilled water	28	11	—
8	10	196 ± 19	4 g chow daily + caffeine[c]	28	100	7

[a] Mean ± SD.
[b] As percentage of controls.
[c] Caffeine was administered orally in a dose of 185 mg/kg/day.
[d] Expressed as mean ± SD of mean daily food intake in grams.

The experiments were continued for 14 days. The survivors were then killed with chloroform and autopsied. Eighteen organs, listed in Table 2, were weighed and the water content of portions was determined by drying to constant weight at 96°C in a forced draft oven.

The statistical methods used were those of Croxton (1959).

RESULTS

Mortality rates in the various groups are listed in Table 1. Reducing food intake in groups 6 and 8 to 43 and 28% of ad libitum intake, respectively, increased the susceptibility to caffeine. One rat of group 7 died from reduction of food intake alone.

Two types of death occurred. Type 1 ("hemorrhagic death") followed automutilation and extensive blood loss; it was most frequent during the first week. Type 2 ("starvation death") followed weak tonic-clonic convulsions after a period of

gradually increasing weakness. Type 2 was similar to that seen after starvation alone in group 7 and occurred at a loss of 35-40% of initial body weight. A body weight gain was often noticed on the day before type 2 death, apparently due to a premortal water retention.

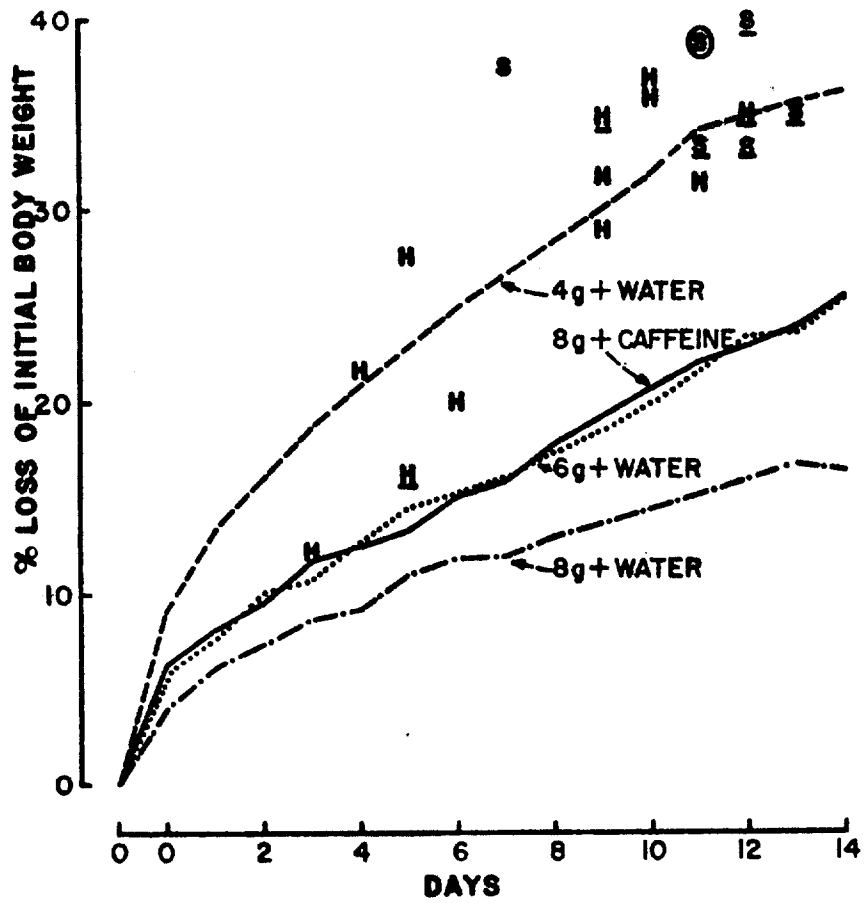

FIG. 1. The mean percentage loss of initial body weight in survivors of groups 3, 4, 5, and 7, and individual percentage loss at death in nonsurvivors. *H* indicates loss from hemorrhagic death, and *S* indicates death from starvation. Unmarked letters indicate deaths in rats on 4 g of chow and caffeine, underlined letters indicate deaths in rats on 6 g of chow and caffeine, and the one encircled point represents a rat on 4 g of chow and distilled water.

Body weight was not diminished by caffeine administration alone (group 2) except for some loss during the first 2 days. Loss of body weight in the remaining animals is shown in Fig. 1. It is obvious that caffeine administration did augment loss of body weight when food intake was reduced below ad libitum amounts. For example, at 14 days the mean ± SD loss in group 4 was 25.7 ± 7.3%, which is significantly ($P < 0.01$) higher than the loss of 16.4 ± 4.2% which occurred in group 3.

Mean changes in water intake and urinary volume are shown in Fig. 2. As body

weight declined during the second week in rats on starvation diets, caffeine diuresis declined. During the first week on starvation diets, caffeine diuresis was temporarily augmented, particularly in group 6 which had a significantly ($P < 0.001$) higher urinary volume on days 3 and 4 than had group 2. Shifts in water intake more or

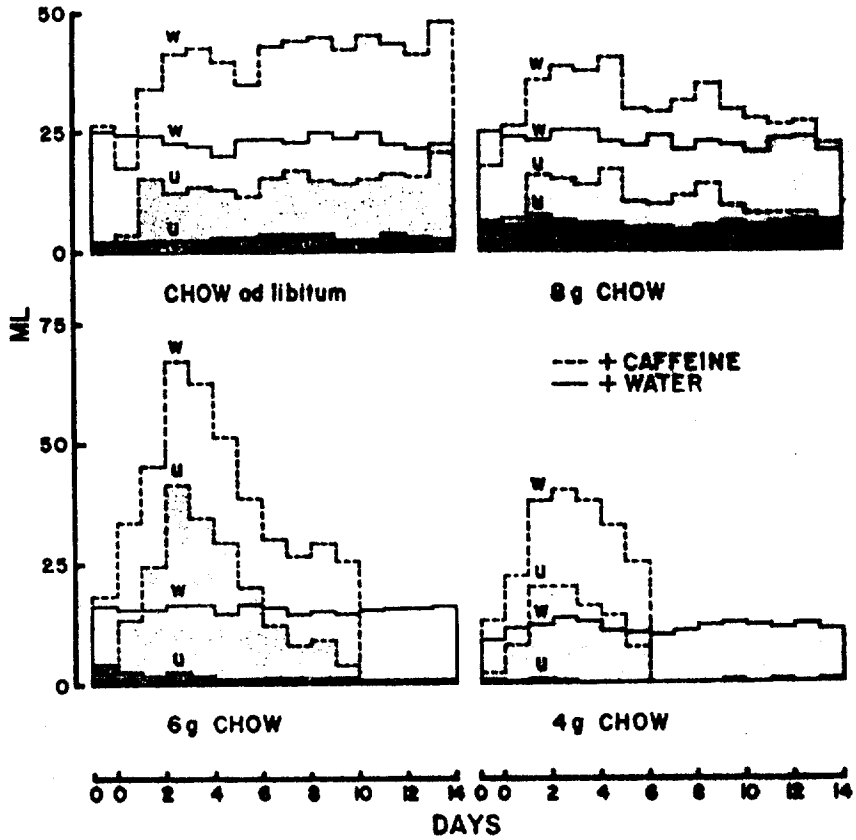

FIG. 2. Mean daily water intake (W) and urinary volume (U) of rats given varying daily amounts of chow with caffeine (interrupted line) or distilled water (solid line).

less paralleled the changes in urinary volume. The quadratic regression of water intake and urinary volume on food allowances at day 3 in individual rats of groups 4, 6, and 8 is illustrated in Fig. 3.

Caffeine diuresis during the initial 3 days was accompanied by a glycosuria which was not influenced by starvation. Caffeine diuresis was associated with a slight alkalinuria and no change in urinary protein output, and these were not affected by starvation.

Results for organ weights and water levels of fortnight survivors in unstarved rats of groups 1 and 2 are summarized in Tables 2 and 3. Changes in organ weight were similar to those reported by Boyd *et al.* (1965) except that lung weight was not increased as it was, due to development of bronchopneumonia, in the longer daily administration of caffeine by Boyd *et al.* (1965). The significant dehydration

of several organs noted in Table 3 did not occur in the work of Boyd *et al.* (1965), who found slight hydration of most organs after their more prolonged daily administration of caffeine.

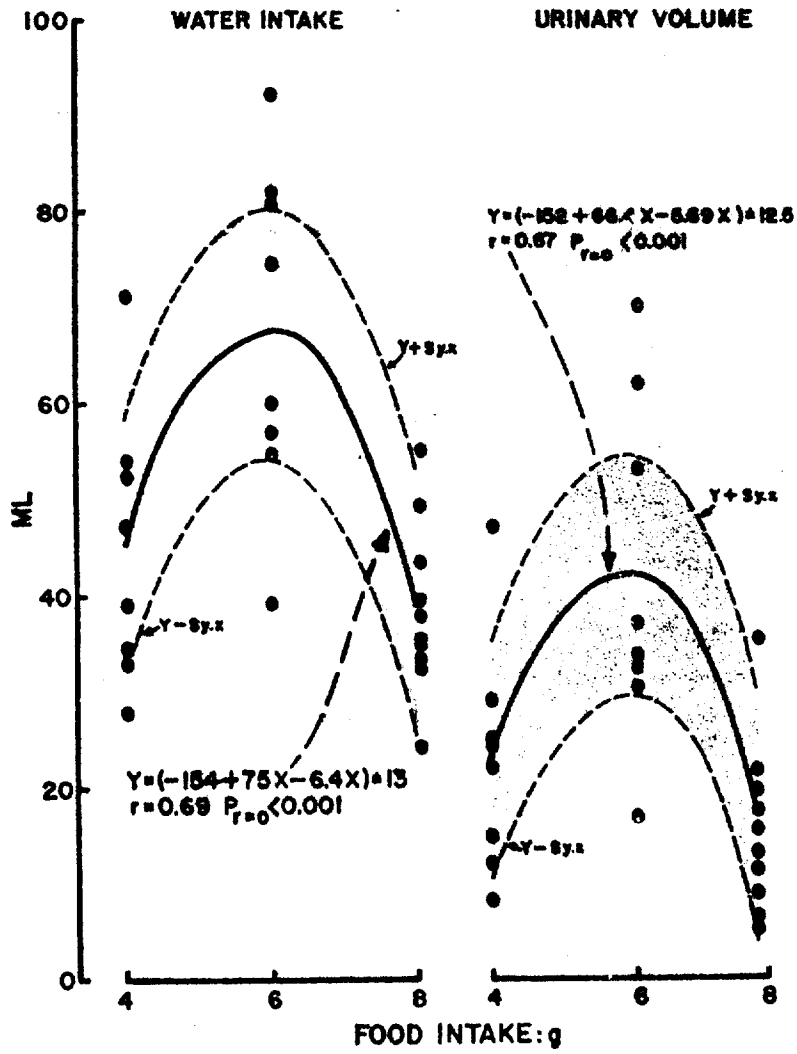

FIG. 3. The regression at day 3 of water intake and urinary volume on food allowance of albino rats given caffeine orally in a dose of 185 mg/kg/day.

Organ weights and water levels of survivors in group 4 have been compared with controls given water and the same amount of food (group 3) and with controls which lost the same amount of body weight (group 5) in Tables 2 and 3. It may be seen that the main influence of this degree of starvation on caffeine toxicity was the production of a relative increase in the weight of the ovaries and the thymus gland (compare especially columns 1 and 3 of Table 2). The corresponding effect on organ water levels (Table 3) was a more or less generalized tendency to hydration of tissues

as reported in unstarved rats given daily caffeine for a longer period of time by Boyd *et al.* (1965).

Hair loss was present in 50, 37.5, 20, and 0% of the rats in groups 8, 6, 4, and 2, respectively. Hair loss can occur in an occasional rat after 2-3 months of daily caffeine administration on chow ad libitum (Boyd *et al.*, 1965). It is obvious that increasing starvation produced an increasing incidence of this toxic sign.

DISCUSSION

Food intake obviously has to be reduced to less than half ad libitum amounts before the susceptibility of rats to caffeine toxicity increases appreciably. The

TABLE 2
ORGAN WEIGHTS OF RATS FED LABORATORY CHOW AD LIBITUM AND IN RESTRICTED AMOUNTS, WITH AND WITHOUT DAILY CAFFEINE

Organ	Caffeine + chow ad libitum compared with water plus chow ad libitum[a] (%)	Caffeine + 8 g chow daily compared with water plus 8 g chow daily[a] (%)	Caffeine + 8 g chow daily compared with water plus 6 g chow daily[a] (%)
Adrenal glands	+44.4[c]	+40.4[c]	+29.5[c]
Brain	− 0.7	+13.8[c]	+ 3.8
Gastrointestinal tract			
Cardiac stomach	+12.3[c]	+35.3[c]	+24.3[c]
Pyloric stomach	+12.0[b]	+ 0.8	+ 1.5
Small bowel	+18.6[c]	+18.2[c]	+18.7[c]
Cecum	+ 8.0	−16.0[b]	+ 5.1
Colon	+10.2	+24.0[c]	+16.3[c]
Heart	+12.0[c]	+ 4.0	+ 1.0
Kidneys	+ 7.0[b]	+18.0[c]	+12.0[c]
Liver	+18.8[c]	+19.0[c]	+22.4[c]
Lungs	− 9.9	+ 7.4	+ 7.0
Muscle (ventral abdominal wall)	− 2.3	−14.2[c]	− 7.4
Ovaries	+ 8.4	+51.1[c]	+95.6[c]
Skin	− 8.4[c]	− 3.1	− 1.3
Spleen	+14.8	+22.1[b]	+22.4[b]
Submaxillary salivary glands	+76.3[c]	+37.4[c]	+61.5[c]
Thymus	−63.3[c]	−40.1[c]	− 4.9
Residual carcass	− 2.5	− 2.6	− 6.5[c]
Body weight	− 1.4	−14.2[c]	− 3.2

[a] Changes in organ weight are expressed as mean percentage change from control animals given distilled water. Organ weights were calculated as per cent of body weight at autopsy.

[b] Significantly different from control, $P = 0.05$-0.02.

[c] Significantly different from control, $P = 0.01$ or less.

effect of partial starvation is relatively less significant than that produced by change of diet (Peters and Boyd, 1966) or by old age (Peters and Boyd, 1964). Therefore, partial starvation unless it approaches no food intake may be classified as a minor factor influencing caffeine toxicity. It was not likely to have caused increased susceptibility to caffeine in animals with pathologic conditions reported by Salant and Rieger (1912).

Mortality was due either to caffeine-induced automutilation aggravated by starvation or to starvation aggravated by caffeine administration. There appeared to be an increased need for calories only when calories were extensively restricted in the diet. This might be associated with heat loss due to an increased metabolic rate

TABLE 3
CHANGES IN WATER CONTENT OF ORGANS OF RATS FED LABORATORY CHOW AD LIBITUM AND IN RESTRICTED AMOUNTS, WITH AND WITHOUT DAILY CAFFEINE

Organ	Caffeine + chow ad libitum compared with water plus chow ad libitum[a] (%)	Caffeine + 8 g chow daily compared with water plus 8 g daily[a] (%)	Caffeine + 8 g chow daily compared with water plus 6 g daily[a] (%)
Adrenal glands	−3.81[c]	+4.52[c]	+1.51
Brain	−0.33[b]	+0.12	+0.52[c]
Gastrointestinal tract			
Cardiac stomach	+1.57[c]	+1.70[c]	+1.44[c]
Pyloric stomach	+0.49	+1.65[b]	+0.40
Small bowel	+0.02	+2.90[c]	+2.00
Cecum	−1.99[c]	+1.74[c]	+2.36[c]
Colon	+0.10	+1.56[c]	+1.56[c]
Heart	+0.03	+0.93	+0.76
Kidneys	+1.00[c]	+1.03	+1.54[b]
Liver	+0.56	+1.40[c]	+0.47
Lungs	−0.16	+0.48	+1.06[c]
Muscle (ventral abdominal wall)	−0.03	+1.14[b]	+0.61
Ovaries	−1.91[b]	+0.03	+2.17
Skin	−0.36	+2.09	+2.92[c]
Spleen	−0.18	−0.40	+0.15
Submaxillary salivary glands	−2.29[c]	+0.02	+0.04
Thymus	−2.04[c]	+1.82[b]	+2.62
Residual carcass	+0.25	−0.07	−0.30

[a] Changes in organ water content are expressed as mean percentage change from control animals given distilled water.
[b] Significantly different from controls, $P = 0.05$-0.02.
[c] Significantly different from controls, $P = 0.01$ or less.

after caffeine administration (Truitt, 1965). Caffeine could overantagonize the decrease in basal metabolism produced by starvation alone (Friedman, 1966). Boyd *et al.* (1965) reported no significant change in the body temperature of rats given caffeine in a similar daily dosage range.

Caffeine may have interfered with optimal digestion of the reduced amounts of food. Caffeine has been shown to affect the motility of the gastrointestinal tract (Truitt, 1965). Boyd (1959) described intussusception and Boyd *et al.* (1965) increased weight of the gastrointestinal tract.

The diuretic effect of caffeine was augmented in the first week in rats with the available food supply reduced to some 50%. Thereafter diuresis was reduced. Glycosuria after caffeine administration has been previously reported by Salant and Rieger (1912) and by Boyd *et al.* (1965).

Organ weight changes in groups fed ad libitum are in general agreement with

those reported by Boyd *et al.* (1965). Partial starvation (8 g of chow per day) produced a relative hypertrophy of the ovaries and thymus gland which was not seen in the experiments of Boyd *et al.* (1965).

Boyd *et al.* (1965) reported hydration of many organs after the prolonged daily administration of caffeine. This was not seen after fortnight administration of caffeine in the present work but did appear in partially starved rats. In this respect, starvation augmented caffeine toxicity.

SUMMARY

The effect of increasing partial starvation upon caffeine toxicity was studied in albino rats. The daily available food was reduced by 0, 43, 57, and 72% of that in animals fed ad libitum, and caffeine was given orally in a daily dose of 185 mg/kg for 14 days. At a 43% reduction of food intake, there was some loss of body weight in excess of that in pair-fed controls, and some hydration of the tissues but no increase in mortality and other signs of caffeine toxicity. Only when food intake was reduced to less than half normal were signs of caffeine toxicity markedly increased. Signs in the latter animals included augmented loss of body weight, marked diuresis followed by a relative oliguria, hair loss, and death due to automutilation or cachexia. Starvation did not alter the temporary initial glycosuria and alkalinuria produced by caffeine. Starvation, therefore, is not a factor of great significance in caffeine toxicity, as measured, until daily food allowance is reduced by over 50%.

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REFERENCES

BOYD, E. M. (1959). The acute oral toxicity of caffeine. *Toxicol. Appl. Pharmacol.* **1**, 250-257.

BOYD, E. M., DOLMAN, M., KNIGHT, L. M., and SHEPPARD, E. P. (1965). The chronic oral toxicity of caffeine. *Can. J. Physiol. Pharmacol.* **43**, 995-1007.

CROXTON, F. E. (1959). *Elementary Statistics with Applications in Medicine and the Biological Sciences.* Dover, New York.

DIXON, R. L., SHULTICE, R. W., and FOUTS, J. R. (1960). Factors affecting drug metabolism by liver microsomes. IV. Starvation. *Proc. Soc. Exptl. Biol. Med.* **103**, 333-335.

FRIEDMAN, L. (1966). Nutritional status and biological response. *Federation Proc.* **25**, 137-144.

PETERS, J. M., and BOYD, E. M. (1964). Secondary factors in caffeine toxicity. *Proc. Can. Federation Biol. Soc.* **7**, 37.

PETERS, J. M., and BOYD, E. M. (1966). Diet and caffeine toxicity in rats. *Toxicol. Appl. Pharmacol.* **8**, 350-351.

SALANT, W., and RIEGER, J. B. (1912). The toxicity of caffeine: an experimental study on different species of animals. *U.S. Dept. Agr. Bur. Chem. Bull.* **148**, 5-98

TRUITT, E. B. (1965). Analeptic and convulsive drugs: The xanthines. In: *Drill's Pharmacology in Medicine* (J. R. DiPalma, ed.), 3rd ed. pp. 394-409. McGraw-Hill, New York.

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CAFFEINE-INDUCED HEMORRHAGIC AUTOMUTILATION (1)

BY

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Psychotic-like reactions in caffeine-intoxicated rats were observed by BOYD *et al.* (1), who noticed selfmutilation in the form of the animal biting and mutilating its own feet or tail at the end of one month's drug administration. Earlier, PFEIFFER and GASS (2) had briefly mentioned selfmutilation in rats receiving 100 mg/kg caffeine but these rats had been starved concomitantly. BOYD (3) did not report selfmutilation in rats given single lethal doses of caffeine. Automutilation is not reported in man (4) but MCMANAMY and SCHUBE (5) described "wild, manic screaming, kicking and biting." The present report is of a study on the incidence of caffeine-induced automutilation in the albino rat and of factors which affect the incidence.

MATERIALS AND METHODS

The animals used were male and female albino rats of a Wistar strain which had been bred in the animal quarters of the department. They were fed laboratory chow (3) and water ad libitum and were segregated in metabolism cages, one rat per cage, except as noted below. When aggregated, the maximal number was ten in a cage, 55 cm wide, 75 cm deep and 40 cm high. Details of treatment in the various groups are summarized in Table I.

(1) This project was supported by a grant from the Medical Research Council of Canada. The author wishes to express his gratitude to Prof. Eldon M. BOYD for professional advice and to Tina KRIJNEN for technical assistance.

(2) Fellow, Medical Research Council of Canada.

(3) Obtained as Purina Laboratory Chow Checkers, Ralston, Purina Company, Limited, Woodstock, Ontario, Canada.

TABLE I

The incidence of automutilation at two weeks in albino rats given oral caffeine in a dose of 185 mg/kg per day

Factor Studied	Group Number	Sex	Body weight (g)	Treatment	Number of animals (1)	Per cent Mortality	Per cent Automutilated (2)
Diet	1	F	130–160	chow	12 (17)	0	8
	2	F	130–160	chow	18 (17)	33	33
	3	F	130–160	diet 1	27 (10)	70	44 (1)
	4	F	130–160	diet 2	29 (10)	83	76 (1, 2)
Starvation	5	F	180–210	4 g chow/rat	10 (9)	100	90 (7, 8)
	6	F	180–210	6 g chow/rat	8 (6)	88	38 (7, 8)
	7	F	180–210	8 g chow/rat	10 (8)	0	0
	8	F	180–210	ad lib chow	9 (10)	0	0
Cachexia	9	F	130–160	rancid diet 1	54 (17)	53	50 (1)
Age and sex	10	F	90–110	chow	10 (10)	0	0
	11	F	250–300	chow	13 (10)	0	0
	12	M	110–130	chow	19 (10)	0	21 (10)
	13	M	310–370	chow	13 (9)	0	31 (8)
	14	M	390–500	chow	13 (8)	0	31 (11)
Aggregation	15	F	130–160	chow	21 (17)	5	0 (2)
	16	F	250–300	diet 1	5 (10)	100	20
	17	F	130–160	diet 2	20 (10)	50	5 (4)
	18	F	130–160	rancid diet 1	25 (17)	24	20 (9)

(1) Number of control animals given no caffeine is given in parenthesis after number treated with caffeine. — (2) The control group or groups from which the automutilation incidence was significantly different at $P = 0.05$ or less is indicated in parenthesis.

Caffeine was administered as caffeine alkaloid monohydrate, U.S.P., in a dose of 185 mg/kg daily for 14 days by intragastric tube, dissolved in distilled water to a volume of 20 ml per kg body weight. At this dosage level, mortality at 14 days is usually zero and rarely exceeds 10 %. Controls were given 20 ml/kg of distilled water daily. Automutilation was recorded as present when self-biting produced hemorrhage. The significance of differences in frequency was tested by the chi-square method with Yates' correction (6).

The effect of caffeine was tested in rats fed laboratory chow, Biotin Deficient Rat Test Diet (1) (Diet 1) and Vitamin B Complex Diet — Complete (2) (Diet 2). Starvation was induced by reducing chow intake to 4, 6 and 8 g per rat daily, and caffeine was given one hour after feeding. Caffeine was also given to rats which developed cachexia by being fed rancid diet 1 which reduced their voluntary food intake. Caffeine was administered to groups of female rats of 1.5 (group 10), 2.5 (groups 1 and 2), 4.5 (group 8) and 12 (group 11) months of age and to male rats of 1.5 (group 12), 4.5 (group 13) and 12 (group 14) months of age.

Aggregation was studied in groups 15 to 18. Simultaneously with group 2, 21 female rats, were aggregated in 3 subgroups and given laboratory chow and caffeine (group 15). Five female rats were aggregated and given diet 1 and caffeine (group 16). Group 17 consisted of 20 female rats aggregated in 2 subgroups and given diet 2 and caffeine. Group 18 contained 30 female rats aggregated in groups of 2 and fed rancid diet 1 and caffeine. Except in group 16, the aggregated rats were littermates of their respective segregated controls. Caffeine administration was extended to 30 days in 10 rats of group 15, then they were segregated one per cage and caffeine given for a further 10 days with the 12 rats of segregated group 1 carried as controls for 40 days.

Results

For several hours after the first administration of caffeine, activity of the rats was decreased. They appeared listless, drowsy and their eyes were half closed and moist. There was occasional teeth-chattering. Thereafter the rats developed signs such as licking, fumbling quickly with the forelegs, running backwards or in circles, climbing and tail chasing. In some rats this last feature progressed to biting and mutilating the high legs and the end, middle or root of the tail as shown in Fig. 1. Mutilation of forelegs, knees and penis was rare. Automutilation was so acute and intense in some rats that the animals died from hemorrhagic shock: blood taken from the heart contained 4 g or less hemoglobin per 100 ml.

A ball of wire put in the cage served as a temporary substitute for

(1) Obtained as Biotin Deficient Rat Test Diet from General Biochemicals, Chagrin Falls, Ohio. Composition: Dried egg white 30 %, Corn starch 48 %, Cottonseed oil 14 %, Cod liver oil 3 %, Salt mix, Phillips-Hart IV 5 %, and complete vitamin supplementation except biotin.

(2) Obtained as Vitamin B Complex Diet-Complete, from Nutritional Biochemicals Corporation, Cleveland 28, Ohio. Composition: vitamin test casein 18 %, sucrose 68 %, vegetable oil 10 %, U.S.P. salt mixture no. 2, 4 %, supplemented with the complete Vitamin diet fortification mixture.

self-aggression but ultimately the animal would turn upon itself. Also attacked were the end of the water bottle or the cage wire. Attacks came intermittently. When biting itself, the animal would shriek and then stop biting. For several minutes it sat motionless, half erect with head lifted, occasionally the teeth chattered and there were rapid, swimming like movements of the fore legs. Externally applied irritation, like pricking, could induce an attack, but when handled, the animals were

FIG. 1

Automutilation of the tail (*a*) and of the hind feet (*b*) in rats given caffeine and diet 2.

not aggressive towards the experimenter. Controls given no caffeine exhibited no automutilation.

In groups 1 and 2 given the standard laboratory chow diet and caffeine automutilation was seen in 8 and 33 %, respectively. Incidence was increased by feeding purified diets 1 and 2 in which automutilation was most frequent at days 3 to 6 but was present throughout the 14 days of treatment.

Starvation increased the incidence of automutilation after reduction of food intake to less than one half ad libitum amounts (groups 5 and 6). Automutilation occurred from the 3rd to 12th day of this experiment.

Feeding rancid diet 1 resulted in a voluntarily reduced food intake and loss of body weight comparable to that in group 6. The additional administration of caffeine (group 9) produced a high incidence of automutilation which was not significantly different to that in group 3 fed fresh diet 1.

Self-aggression was more frequent in male than in female rats but was not significantly affected by age.

Aggregation significantly lessened the frequency of automutilation in groups 15 to 18 over that in their respective controls. When 10 rats of group 15 were segregated on day 30, automutilation increased to 50 %. In group 1, the incidence of automutilation by day 40 had reached 67 %.

Discussion

Automutilation after caffeine appears to be associated with no loss of pain, because after biting the animal shrieks and withdraws the extremity. Aggression is indicated by preliminary tail chasing and the attacking of substitutes or cage mates. An increased aggressiveness in rats after caffeine administration is mentioned by Tonini *et al.* (7).

Synthetic diets 1 and 2 were tried because studies from this department reported in 1963 (8) had demonstrated that such diets augmented susceptibility of rats to caffeine toxicity and that the absence of biotin in diet 1 was not the causative factor. Recent studies indicate that the high level of dried egg white in diet 1 (9) and of sucrose in diet 2 (10) may have been responsible for increased susceptibility of rats to caffeine toxicity when fed these synthetic diets. The incidence of automutilation was increased when rats were fed these diets and simultaneously there was an increase in deaths due to (*a*) convulsions and cardiorespiratory failure and to (*b*) hemorrhagic shock from blood loss due to automutilation.

Starvation was investigated because PFEIFFER and GASS (2) had reported automutilation in starved rats given caffeine and because PETERS (11) had found that the death rate from caffeine is augmented when food intake is reduced to less than half ad libitum amounts. In the latter work, PETERS (11) found an increased number of deaths due to (*a*) hemorrhagic shock secondary to automutilation and (*b*) starvation cachexia but not to convulsions and cardiorespiratory failure.

It would appear that an increased incidence of automutilation is not necessarily associated with an increase in the general toxic reaction to caffeine but only in that component which induces aggression and particularly self-aggression. This distinction was particularly evident in studies of caffeine toxicity versus age of rat. PETERS and BOYD (12) found that the per cent mortality to caffeine is greater, given mg/kg body weight, in older than in younger rats due solely to an increase in the convulsive type of death in the old rats. The incidence of automutilation was not greater in older animals than in younger animals. It is obvious, therefore, that increased incidence of automutilation can result in an increased number of deaths due to blood loss from the mutilated part, i.e. that increase in the toxic death rate is the result, not the cause, of automutilation.

Feeding rancid diet 1 was studied because PETERS and BOYD (13) found that rats on this diet detoxified pentobarbital better than did rats on laboratory chow due to a relative hypertrophy of the liver in the rancid diet-treated group. In spite of the fact that rats on rancid diet 1 lost considerable weight and were cachectic in appearance, they were not more susceptible to automutilation than were rats fed fresh diet 1 and death rates were similar in the two groups.

The incidence of automutilation in males was possibly slightly greater than that in females. PETERS and BOYD (12) found no difference in death rates to caffeine in the two sexes but noted that certain sublethal signs of toxicity, such as loss of body weight, were more evident in males.

Aggregation was investigated because BOYD *et al.* (1) had not reported automutilation a prominent feature in their studies on the chronic oral toxicity of caffeine and had aggregated their rats during treatment. It appeared that the incidence of automutilation should be less in aggregated than in segregated rats and this was found to be so in the results of groups 15 to 18. The high death rates in group 16 was due to convulsive-type deaths in old rats. Nine of the 10 deaths in group 17 were of the convulsive type and the per cent mortalities in groups 17 and 18 were significantly less than in segregated groups 4 and 9 respectively due in

each case to a reduction in deaths following automutilation and hemorrhage.

The mortality results with aggregated animals given caffeine at first sight appear to be opposite to those in aggregated rats given amphetamine in which mortality was originally reported increased by GUNN and GURD in 1940 (14). When rats are aggregated and given daily caffeine, their aggressive behaviour appeared to be satisfied to a large extent by chasing and biting neighbor rats which usually run away before they had been severely mutilated. Thus the effect of aggregation was to enable aggression to be directed mainly at neighbor rats, rather than to automutilation, with a consequent decline in deaths due to automutilation.

Summary

Oral administration of caffeine in a daily dose of 185 mg/kg body weight produced automutilation, severe hemorrhage and eventually death in segregated albino rats. The reaction was due to caffeine-excited aggressive behaviour self-directed mainly because of the absence of neighbor rats. The incidence of automutilation was augmented by feeding synthetic diets containing large amounts of egg white powder or sucrose or by reducing the intake of laboratory chow to less than half ad libitum amounts. The incidence was slightly greater in male than in female rats but was not affected by age or body weight. The incidence of automutilation was markedly reduced when the rats were aggregated.

References

1. — BOYD, E. M., DOLMAN, M., KNIGHT, L. M. and SHEPPARD, E. P. *Can. J. Physiol. Pharmacol.*, 1965, *43*, 995.
2. — PFEIFFER, C. J. and GASS, G. H. *Can. J. Biochem.*, 1962, *40*, 1473.
3. — BOYD, E. M. *Toxicol. appl. Pharmacol.*, 1959, *3*, 250.
4. — TRUITT, E. B. *The Xanthines*, p. 404, in J. R. DiPalma (Ed.): *Drill's Pharmacology in Medicine*, McGraw-Hill, New York, 1965.
5. — MCMANAMY, M. C. and SCHUBE, P. G. *New Engl. J. Med.*, 1936, *215*, 616.
6. — CROXTON, F. E. *Elementary statistics with applications in medicine and the biological sciences*, New York, Dover, 1959.

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THE INFLUENCE OF SEX AND AGE IN ALBINO RATS GIVEN A DAILY ORAL DOSE OF CAFFEINE AT A HIGH DOSE LEVEL[1]

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Caffeine was given daily for 14 days in an oral dose of 185 mg/kg to male and female albino rats in groups of increasing body weight. The sensitivity of rats to the lethal effects of caffeine increased with increase in age. Psychotoxic (automutilation plus hemorrhage) deaths were most common in young rats, and hypokinetic–convulsive deaths in older animals. In survivors, the incidence of glycosuria and hydration of the kidneys was greater, and dehydration of the adrenal glands and gonads less, in older than in younger rats. Sublethal signs of toxicity in male survivors were greater than in female survivors. Anorexia and loss of body weight were greater in male than in female survivors, diuresis was less evident, loss of muscle weight was greater, and the relative gain in weight of the adrenal glands and gastrointestinal tract were greater. In brief, caffeine was found to be more toxic in older than in younger rats and in males than in females.

Introduction

Sex and age of experimental animals are of considerable importance in estimates of drug toxicity (1, 2). Most sex differences are quantitative and can be traced to differences in enzyme activities and rate of metabolism (1). Young animals are often more sensitive to drugs than adults, but there are many exceptions (1).

The significance of sex and age in estimates of caffeine toxicity has not been extensively studied. In 1912 Salant and Rieger (3) reported a series of studies on caffeine toxicity. In 1946 Günther and Navarro (4) found an increase in sensitivity with increasing body weight of female rats. Boyd *et al.* (5, 6) restricted their recent studies on caffeine toxicity to adult female rats. A low dose of aminophylline, a related drug, is advised for children because of their marked sensitivity to the central stimulant action (7). Results obtained in the present investigation may—or may not—have meaningful application to this observed toxicity of aminophylline in children.

The immediate objective of this study was to obtain information on the significance of the sex and age of albino rats used for estimates of the chronic oral toxicity of caffeine by the method of Boyd *et al.* (6).

Methods

The animals used were albino rats of a Wistar strain, which had been bred in the animal quarters of the department. During the experiment, the animals

[1]This project was supported by a grant from the Medical Research Council of Canada.
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[3]The authors thank Tina Krijnen for technical assistance and Damon Card for computer analysis of the data.

were housed separately in metabolism cages and transferred daily to clean ones. Pulverized laboratory chow[4] was offered in a non-spilling feeding device. Body weight, food and water intake, and urine output were measured daily, and the pH, protein, and glucose contents of urine were determined with Ames Combistix reagent strips.

Caffeine was obtained as caffeine alkaloid monohydrate U.S.P. A dose of 185 mg/kg was administered daily by intragastric tube, in a volume of 2.0 ml per 100 g body weight. A daily dose of 150 mg/kg is the $LD_{50(0.1L)}$ of caffeine, i.e. the daily dose which kills 50% of rats when given over a period of 100 days or $\frac{1}{10}$ of the animal's normal life-span (0.1L) as reported by Boyd *et al.* (6). The work described herein was begun before that of Boyd *et al.* (6) was completed and at a point where it appeared that the $LD_{50(0.1L)}$ would be about 185 mg/kg. Control animals were administered orally 2.0 ml distilled water per 100 g body weight per day.

Number, initial body weight, age of the animals, and their treatment are summarized in Table I. The experiments were terminated at 14 days since the daily dose of caffeine had produced appreciable numbers of deaths at this time in certain groups, as noted in Table I. Survivors were then killed with chloroform and autopsied. Weight and water content were determined on the organs listed in Table III. The gastrointestinal tract was cleaned by washing and milking. The organs were dried to constant weight in a Fisher forced-draft Isotemp oven at 96 °C. Suitable aliquots of skin and homogenized carcass were taken for this purpose. All differences between means were subjected to Student's *t* tests of statistical significance, and differences between incidences to chi square tests with Yates' correction (8).

Results

Mortality rates in the various experimental groups are summarized in Table I. Death rates in the 1-year-old males and females were significantly higher than in any of the other groups except group 4, in which the *P* value was not quite 0.05.

The type of death in these older animals was also different. In groups 4, 6, 10, and 12, death followed automutilation and extensive blood loss. In old male rats (group 14) this type occurred in 4 out of 10 deaths, ulceration and perforation of the duodenum were found in one, and the remaining five and all deaths in group 8 (older females) were like acute death from caffeine described by Boyd (5). Before death the animal was prostrate against the floor of the cage, the hind legs spread. Breathing was slow and deep, the eyes were often closed, with marked lacrimation, and the corners of the eyes were red. Salivation was profuse and watery. Soft stool or diarrhea was passed, the latter sometimes whitish, transparent mucus. The rats were sluggish to stimulation and movements were slow and ataxic. Death usually followed mild tonic-

[4]Obtained as Purina Laboratory Chow Checkers, Ralston Purina Company, Limited, Woodstock, Ontario.

TABLE I

Groups, age, body weight, sex, treatment, and mortality rates in rats given caffeine* or distilled water†

Group No.	Age (months)	Initial body weight, mean ± S.D. (g)	Sex	Treatment	Mortality rates at 14 days	
					Incidence	Percent
1	1.5	105±12	F	Water	0/10	0
2	1.5	105±14	F	Caffeine	0/10	0
3	2.5	150±10	F	Water	0/7	0
4	2.5	143±11	F	Caffeine	6/18	33
5	4.5	185±10	F	Water	0/10	0
6	4.5	183±13	F	Caffeine	1/17	6
7	12	275±27	F	Water	0/10	0
8	12	264±28	F	Caffeine	9/13	69
9	1.5	121±10	M	Water	0/10	0
10	1.5	112±12	M	Caffeine	2/19	11
11	4.5	353±18	M	Water	0/9	0
12	4.5	346±33	M	Caffeine	2/13	15
13	12	431±34	M	Water	0/8	0
14	12	447±65	M	Caffeine	10/13	77

*Dose of caffeine was 185 mg/kg per day administered orally in 20 ml/kg of distilled water.
†Dose of distilled water was 20 ml/kg per day administered orally.

clonic convulsions with respiratory failure as the immediate cause. Fifty percent mortality was reached in 2 days in group 8 and in 2 to 3 days in group 14.

Animals which died in group 8 lost 10.1 ± 1.2% of their initial body weight on the first day of the experiment, which was significantly ($P = 0.001$) higher than the loss of 4.0 ± 2.7% in animals which survived until 14 days. On that day their food intake (0.5 ± 0.7 g) was also significantly ($P < 0.001$) lower than that of survivors (7.2 ± 2.7 g). A similar trend was noted in group 14.

Loss of body weight or inhibition of growth in survivors was greater in male than in female rats as shown in Table II. The sex difference was especially obvious in the youngest animals, as illustrated in Fig. 1. Food intake of surviving male rats was depressed to a greater extent (from controls given water) than in surviving females (Table II and Fig. 1). Water intake was increased less (from controls given no caffeine) in male than in female survivors as might be expected from the normal interrelation between food and water intake (9). Caffeine diuresis was relatively more marked in female than in male survivors (Table II and Fig. 1) and in young versus old animals.

The incidence of glycosuria increased with age, and the peak incidence occurred earlier (Table II). Glycosuria was absent in the urine of the controls. The protein content of urine averaged about 100 mg per 100 ml in all controls, and the total daily output was not affected by caffeine since concentration fell in proportion to the diuresis. Urinary pH averaged 6–7 in control groups and rose to 7–8 with increasing caffeine diuresis.

Results for organ weights of the survivors of all groups except 7, 8, 13, and 14 are summarized in Table III. In male survivors, the relative increase in weight of the adrenal glands and in most parts of the gastrointestinal tract

TABLE II

Weight changes, food and water intake, urine volume, and incidence of glycosuria in survivors given caffeine or water

Group No.*	Weight change,† mean ± S.D. (g)	Food intake,‡ mean ± S.D. (g)	Water intake,‡ mean ± S.D. (ml)	Urine volume,‡ mean ± S.D. (ml)	Peak incidence of glycosuria§
1 (WF*a*)	+30± 9	13.7±0.7	23±2	1.2±0.5	0/10
2 (CF*a*)	+39± 4	13.8±1.1	41±5	16.6±2.4	1/10 (4)
3 (WF*b*)	+19± 8	13.2±1.1	24±2	3.1±0.7	0/7
4 (CF*b*)	+15± 5	13.0±0.6	48±3	26.9±3.7	5/17 (4)
5 (WF*c*)	+ 7± 6	14.1±0.7	23±1	3.0±0.5	0/10
6 (CF*c*)	+14± 6	14.8±0.8	53±4	27.8±4.9	8/17 (2)
7 (WF*d*)	−13± 6	13.1±1.1	23±1	5.3±0.7	0/10
8 (CF*d*)	− 4±12	18.8±1.2	48±6	21.5±5.3	7/13 (1)
9 (WM*a*)	+46± 8	17.2±1.4	28±3	1.1±0.3	0/10
10 (CM*a*)	+16±15	12.9±0.9	34±4	9.1±2.0	1/19 (2)
11 (WM*c*)	0± 2	19.5±1.3	32±2	9.1±0.8	0/9
12 (CM*c*)	− 3± 2	18.5±1.5	55±3	30.6±3.2	3/12 (3)
13 (WM*d*)	0± 4	22.1±1.4	44±2	15.3±1.2	0/8
14 (CM*d*)	− 6± 3	21.0±2.2	57±7	28.0±6.8	7/13 (1)

*Treatment is indicated in parentheses as follows: W = water; C = caffeine; F = females; M = males; *a* = 1.5 months old; *b* = 2.5 months; *c* = 4.5 months; and *d* = 12 months; for example, in group 12 caffeine was given to males 4.5 months old.

†Change in body weight (g) during the total experimental period.

‡Calculated as the mean of the mean daily measurements during the last 10 days of the experiment.

§The day of peak incidence is indicated in parentheses.

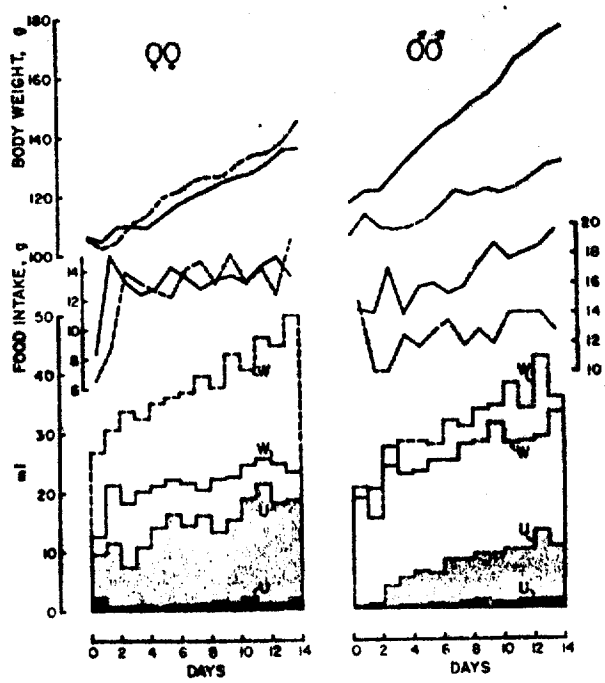

FIG. 1. Mean daily changes after control day zero in body weight, food intake, water intake (W), and urinary volume (U) of young male and female rats with (broken lines) and without (solid lines) caffeine administration. The animals were 1.5 months old at day zero.

TABLE III

Organ weights† of rats given caffeine daily for 14 days‡

Organ	Female rats			Male rats	
	1½ months	2½ months	4½ months	1½ months	4½ months
Adrenal glands	+14.0	+29.6**	+35.6**	+113.5**	+65.9**
Brain	− 7.3	+ 7.8	− 5.0	+ 39.8**	+ 3.1
Gastrointestinal tract					
Cardiac stomach	+ 6.8	+31.9**	+ 0.2	+ 31.4**	+19.1*
Pyloric stomach	+12.4**	+16.2**	+ 4.7	+ 26.9**	+ 7.4
Small bowel	− 4.0	+ 0.3	− 1.7	+ 18.9**	+ 7.4
Cecum	− 3.9	+ 3.7	−11.6	+ 25.4*	+ 6.0
Colon	+ 3.2	− 9.8	+ 6.9	+ 13.1	+10.8
Heart	− 3.0	+ 8.7*	+ 3.4	+ 17.1**	+ 7.7
Kidneys	− 5.0	− 2.1	− 2.3	+ 4.7	+ 2.4
Liver	+22.1**	+13.2**	+ 9.6*	+ 16.2**	+13.4*
Lungs	−16.5**	−15.3	−15.9	+ 6.1	−15.2
Muscle (abdominal wall)	−13.5**	− 9.6*	− 5.0	− 24.4**	−15.5**
Ovaries	− 8.8	− 9.7	−11.6		
Skin	−13.3**	− 6.7*	−10.4**	− 17.4**	− 8.4**
Spleen	−40.4**	+ 9.3	−17.8	− 43.8**	+ 6.2
Submaxillary glands	+53.6**	+60.0**	+44.8**	+139.6**	+61.0**
Testes				+ 10.3*	−10.1
Thymus	−51.0**	−58.9**	−75.3**	− 73.5*	−64.8**
Residual carcass	− 2.4	+ 0.6	− 5.0**	− 5.1**	− 2.8
Body weight	+ 2.9	− 9.7	+ 5.0	− 28.9**	− 6.8
Body weight, controls (g)	136.5	178.0	202.2	176.4	357.0

†Organ weight is calculated as percentage of autopsy body weight.
‡The results are expressed as mean percentage change from respective control animals not given caffeine. Means that differed at $P = 0.05$–0.01 from corresponding means for controls are indicated by one asterisk, at $P \leq 0.01$ by two asterisks.

was greater than in female survivors. Loss of muscle weight was greater in male than in female survivors.

Corresponding changes in organ water content are summarized in Table IV. The only significant difference due to sex was that dehydration of the thymus gland was greater in male than in female survivors. Dehydration of the adrenal glands and the gonads was less, whereas hydration of the kidneys was greater, in the older than in the younger animals.

Discussion

The death rate is higher in old rats than in young rats given daily oral administrations of 185 mg/kg caffeine. Death occurred mainly during the first 2–3 days. Mortality in males was not greater than in females. The results for age agree with the findings of Salant and Rieger (3) for dogs, cats, and rabbits. Age rather than body weight seems to be the determining factor, as suggested by Günther and Navarro (4). It should be noted that the oldest rats used in this study were 1 year old or one-third the rat's normal life-span. No data were obtained on what would correspond to geriatric dose effects.

Weight and food intake are considered sensitive indicators of an animal's response to a drug (2). This was confirmed by the observation that older

TABLE IV

Organ water contents† of rats given caffeine daily for 14 days‡

Organ	Female rats			Male rats	
	1½ months	2½ months	4½ months	1½ months	4½ months
Adrenal glands	−3.13**	−2.74*	−1.66	−3.78**	−1.71*
Brain	−0.58*	+0.11	−0.38*	−0.39**	−0.10
Gastrointestinal tract					
Cardiac stomach	−1.08	+2.16**	−0.01	+0.82	−0.02
Pyloric stomach	−0.40	+0.74	+0.18	+0.25	+0.28
Small bowel	+0.41	+1.43**	+1.21*	+1.22**	+1.13
Cecum	+0.34	−1.84	+0.87	+1.02*	+0.98*
Colon	+0.38	+0.19	+1.00**	+1.18*	+1.22*
Heart	−0.59*	0.00	+0.56	−0.66**	+0.77
Kidneys	−0.54	−0.45	+1.66**	+0.27	+0.90*
Liver	−1.96**	+0.69	+0.50	−0.00	−0.31
Lungs	−0.92*	−0.50	−0.18	−0.56	−0.71*
Muscle (abdominal wall)	−0.45	+0.28	+0.14	−0.02	+0.30
Ovaries	−3.30**	−2.42**	−1.65*		
Skin	−2.42	+3.29	+1.32	+2.09*	−0.29
Spleen	+0.07	−0.08	+0.36	+0.40	+0.04
Submaxillary glands	−0.69	−3.32**	+0.49	−2.16**	−0.78
Testes				−0.48	+0.09
Thymus	−1.79**	−1.40	−1.81*	−3.20*	−8.08**
Residual carcass	−1.71	−0.72	+1.28	−1.53	−2.33

†Measured as percentage wet weight.
‡Expressed as mean percentage change from respective control animals not given caffeine. Means that differed at $P = 0.05$–0.01 from corresponding means for controls are indicated by one asterisk, at $P \leq 0.01$ by two asterisks.

animals which died, ate less and lost more weight after the first administration of caffeine, than did those which survived.

Do sex and age influence the diuretic action of caffeine at this dosage level? This depends on how the results for urinary volume are expressed. When taken as absolute amount, the young animals are less sensitive and no clear difference exists between males and females. When diuresis is expressed as percentage change from respective controls, or the urine volume calculated as milliliters per 100 g body weight, young animals are found more sensitive than older ones and females more than male rats. An absence of animals which died may have affected results in survivors of the 1-year-old groups, but the sex and age differences were present in the remaining groups, in which mortality was much lower. The incidence of glycosuria is greater in older animals.

Increased weights of adrenal glands, liver, and submaxillary salivary glands, and decreased weight of the thymus after caffeine administration confirm the results of Boyd *et al.* (6). Adrenal weight increase and thymic atrophy are parts of the general adaptation syndrome, and Selye (10) includes atrophy of the spleen. In our results splenic atrophy occurred only in young animals. If adrenal hypertrophy is taken as an index of the degree of stress, stress was present more in male than in female rats.

Hypertrophy of the liver after caffeine administration is a reaction also reported for other drugs and has been considered to indicate a stimulation of microsomal enzyme formation (11).

References

1. G. ZBINDEN. Advan. Pharmacol. **2**, 1 (1963).
2. G. E. PAGET and J. M. BARNES. Toxicity tests. Chap. 6 *in* Evaluation of drug activities: pharmacometrics. *Edited by* D. R. Laurence and A. L. Bacharach. Academic Press, New York. 1964. p. 135.
3. W. SALANT and J. B. RIEGER. The toxicity of caffeine: an experimental study on different species of animals. U.S. Department of Agriculture, Bureau of Chemistry. Bull. No. 148. Government Printing Office, Washington, D.C. 1912.
4. B. GÜNTHER and Y. NAVARRO. Rev. Soc. Arg. Biol. **22**, 35 (1946).
5. E. M. BOYD. Toxicol. Appl. Pharmacol. **1**, 250 (1959).
6. E. M. BOYD, M. DOLMAN, L. M. KNIGHT, and E. P. SHEPPARD. Can. J. Physiol. Pharmacol. **43**, 995 (1965).
7. J. R. DIPALMA (*Editor*). Drill's pharmacology in medicine. 3rd ed. McGraw-Hill Book Co., Inc., New York. 1965. p. 404.
8. F. E. CROXTON. Elementary statistics with applications in medicine and the biological sciences. Dover Publications, Inc., New York. 1959.
9. J. L. STROMINGER. Yale J. Biol. Med. **19**, 279 (1948).
10. H. SELYE. J. Clin. Endocrinol. **6**, 117 (1946).
11. A. H. CONNEY and J. J. BURNS. Advan. Pharmacol. **1**, 31 (1962).

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The Influence of a Cachexigenic Diet on Caffeine Toxicity

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Previous studies on factors which influence the subacute toxicity of caffeine demonstrated that when rats are changed from a standard chow diet to other types of diet they may become more sensitive to caffeine toxicity (Peters and Boyd, 1966a). Reduction of the available amount of standard chow diet increased mortality to caffeine only when food intake was less than half the voluntary intake (Peters, 1966).

An opportunity was offered to further evaluate these two factors—change of diet and reduced food intake—when one of the experimental diets tested (Peters and Boyd, 1966a) became rancid after exposure to room temperature. The diet in question was a biotin-deficient raw egg white diet which, after 1 to 2 months of exposure, induced a severe cachexia with reduced food intake, diarrhea, diuresis, and marked loss of body weight. It was anticipated that the debilitated adult rats fed this rancid diet would be more sensitive to the toxic effects of caffeine than animals fed fresh biotin-deficient raw egg white diet which produced almost normal growth in adult rats. The opposite result was obtained.

METHODS

The animals used were female albino rats of a Wistar strain which had been bred in the animal quarters of the department and raised on a diet of laboratory chow[1] and water ad libitum. At the beginning of the experiment their body weight was 148 ± 11 (mean ± SD) g and they were divided into 7 groups as indicated in Table 1.

Caffeine was administered by intragastric cannula daily for 6 days a week in a dose of 185 mg per kilogram of body weight, freshly dissolved in 20 ml of distilled water per kilogram. Controls of groups 5, 6, and 7 were given the same volume of distilled water per kilogram. The dose of caffeine was slightly higher than that which killed 50% of animals after 100 days of administration as reported by Boyd *et al.* (1965). Administration was continued for two months or until all the animals of one comparison group had died, whichever occurred first. This was done upon small numbers of weight-paired animals per group and repeated to the total numbers indicated in Table 1.

[1] Purina Laboratory Chow Checkers, Ralston Purina Company, Limited, Woodstock, Ontario, Canada.

The rancid diet fed to animals of groups 1, 2, 5, and 6 had been stored at room temperature for 4 to 14 months. The toxicity of this diet had reached a maximum at or some time before the 4 months of storage and remained at this maximum during subsequent storage. When purchased[2] it contained 30% of spray-dried raw egg white powder, 48% of cornstarch, 14% of cottonseed oil, 3% of cod liver oil, 5% of salt mix (Phillips-Hart IV), and all vitamin supplements except biotin. Animals of groups 2 and 6 received daily supplements of d-biotin in doses of 5 (adequate) or 200 (massive) μg/kg orally or subcutaneously; since there were no significant differences between the dose subgroups, the results were combined as indicated in Table 1.

TABLE 1

GROUPS OF ANIMALS, TREATMENTS, AND MORTALITY RATES[a]

Group No.	Number of animals	Treatment	Mortality rate[b] (%)	Days to 50% mortality
1	54	Caffeine Rancid diet	89	11
2	30	Caffeine Rancid diet Biotin supplements	97	9
3	36	Caffeine Laboratory chow	8	—
4	16	Caffeine Laboratory chow calorie pair-fed to group 1	5	—
5	46	Distilled water Rancid diet	4	—
6	24	Distilled water Rancid diet Biotin supplements	0	—
7	15	Distilled water Laboratory chow	0	—

[a] Caffeine was administered daily, 6 days a week, in a dose of 185 mg/kg dissolved in distilled water, 20 ml/kg, to groups 1–4; groups 5–7 received 20 ml of distilled water per kilogram daily for 6 days a week.

[b] The mortality rates in groups 1 and 2 were significantly ($P < 0.001$) higher than those of groups 3–7. The percent deaths in group 2 was insignificantly different from that in group 1.

Each rat was placed singly in a metabolism cage for clinical measurements of body weight, food and water intake, colonic temperature, and urinary volume, pH, glucose and protein.[3] Measurements were made at intervals of 1–7 days as indicated.

Since food intake was reduced in rats fed the rancid diet, a group 4 was

[2] Obtained as Biotin Deficient Rat Test Diet from General Biochemicals, Chagrin Falls, Ohio.

[3] The last three measurements were determined with Ames Combistix®, Ames Company of Canada, Limited, Toronto, Ontario, Canada.

introduced in which rats were given caffeine and their ration of laboratory chow was reduced to be calorie-equivalent to that of the corresponding animals in group 1. For this purpose, 0.73 g of the rancid diet was calculated to be calorie-equivalent to 1.00 g of laboratory chow.

Autopsies were performed upon moribund rats or upon rats which had been dead for not over 1 hour. Some of the animals died from hemorrhagic shock due to violent automutilation and they were excluded from the reported autopsy data. Representative animals of the control groups were chloroformed and autopsied at the same intervals. Wet weight and water content were measured on organs listed in Table 2. The contents of the gastrointestinal tract were removed by a standardized process of washing and milking. The organs were dried[4] to constant weight at 95°C, suitable aliquots being selected from

TABLE 2

REPRESENTATIVE ORGAN WEIGHTS AND WATER CONTENTS OF RATS FED LABORATORY CHOW OR RANCID DIET AND GIVEN DAILY CAFFEINE[a]

Organ	Weight		Water content	
	Laboratory chow (group 3)	Rancid diet (group 1)	Laboratory chow (group 3)	Rancid diet (group 1)
Adrenal glands	+22.4[c]	+60.0[c]	+0.8	+5.7[c]
Brain	+ 4.4	+30.0[c]	−0.1	0.0
Gastrointestinal tract				
Cardiac stomach	+10.2[b]	+45.5[c]	−0.1	−0.7
Pyloric stomach	+ 6.9	+38.0[c]	+0.3	+2.3[c]
Small bowel	+ 6.7	+45.7[c]	0.0	+5.5[c]
Cecum	+16.1[c]	+36.5[b]	+0.1	+1.7[b]
Colon	+ 5.9	+ 1.5	0.0	+1.4[b]
Heart	+ 1.9	+ 5.1[b]	−0.1	+0.7[b]
Kidneys	− 2.5	+33.5[c]	−0.1	+0.5
Liver	+ 3.4	+48.6[c]	+0.2	−0.4
Lungs	− 3.8	+19.9	−0.1	−0.8
Muscle (abdominal wall)	− 7.3	−38.3[c]	+0.3	+2.2[c]
Ovaries	− 4.1	−21.2[c]	−1.6	−0.1
Skin	− 6.5[c]	−14.1[c]	+1.3	−2.4
Spleen	−17.4	−30.6[b]	−0.4	−0.6
Submaxillary salivary glands	+31.8[b]	+56.1[c]	−0.6	+1.3
Thymus gland	−44.6[c]	−80.3[c]	−0.4	−7.6[b]
Residual carcass	0.0	−10.4[c]	−1.0	+2.5
Body weight	− 3.4	−27.0[c]		

[a] The results are expressed as mean percentage change from control animals given distilled water and laboratory chow ad libitum. Organ weights were calculated as percent of body weight at autopsy and organ water content as percent wet weight.

[b] Significantly different from controls at $P = 0.05$–0.02.

[c] Significantly different from controls at $P = 0.01$ or less.

[4] Forced Draft Isotemp Oven, supplied by Fisher Scientific Company, Limited, Toronto, Ontario, Canada.

the skin and homogenized residual carcass. The statistical methods used were those of Croxton (1959).

RESULTS

It had been originally intended to aggregate the animals on days no clinical measurements were made but when rats of groups 1 and 2 were aggregated, violent fighting occurred after the second administration of caffeine. This did not occur in the other groups, but all animals were thereafter segregated one per cage throughout the experiments.

All rats fed the rancid diet developed diarrhea, anogenital inflammation, soiled dirty greenish fur, piloerection, alopecia, sunken eyes, arched backs and a slow staggering spastic gait. As indicated in Fig. 1, food intake was markedly reduced and there was loss of body weight. In animals not given caffeine, some adaptation occurred after the first few days. Glycosuria appeared in one-third of the animals during the first and second weeks, but there were no significant changes in urinary pH or protein output.

A significantly high death rate occurred in rats given caffeine and fed the

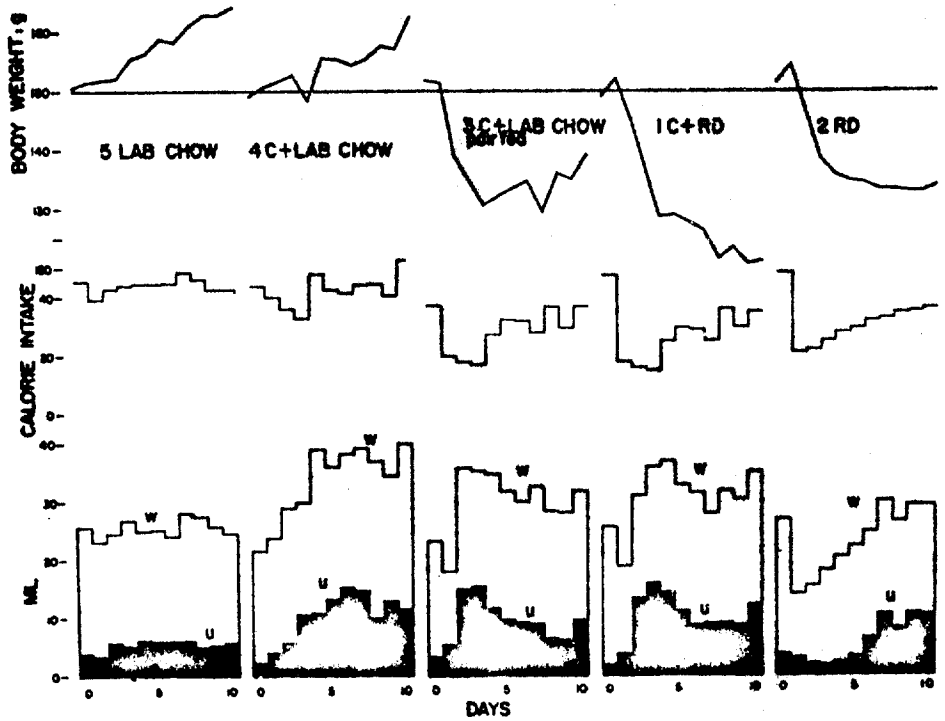

FIG. 1. Mean daily changes in body weight, calorie intake, water intake (*W*), and urinary volume (*U*) of 10-day survivors fed laboratory chow ad libitum and given distilled water daily (group *5 Lab Chow*), fed laboratory chow ad libitum and given caffeine daily (group *4C + Lab Chow*), given laboratory chow calorie pair fed to group 1 plus daily caffeine (group *3C + Lab Chow pair fed*), fed the rancid diet and given daily caffeine (group *1C + RD*), and fed the rancid diet and given distilled water daily (group *2RD*).

rancid diet with or without biotin supplementation as shown in Table 1. Most deaths occurred in the first 2 weeks, but some were delayed to the second month of caffeine administration. One quarter of the deaths were apparently due to marked loss of blood following severe automutilation of the tail, hind limbs, and genital region. One-third of the animals became apathetic and died following respiratory failure in a cachectic hypothermic stupor. Death in the remaining animals followed a mixture of both types of toxic reaction.

Rats of group 3 fed laboratory chow and given caffeine were hyperactive, but there was no excessive aggression or automutilation and the death rate over the period of the experiment was markedly and significantly lower than that in groups 1 and 2 (see Table 1). Caffeine produced a diuresis which augmented water intake, but food intake and growth were similar to those in rats of group 7 fed laboratory chow and given distilled water (see Fig. 1).

Death in group 1 was not due to biotin deficiency since it occurred to the same extent in group 2 given biotin supplementation. The high death rates in groups 1 and 2 were not due to reduced food intake alone since they did not occur in the laboratory chow calorie pair-fed animals of group 4 (see Table 1) in which, however, there was less loss of body weight (see Fig. 1). There were 2 deaths in the 70 rats of groups 5 and 6 fed rancid diet without caffeine and these two animals exhibited the lethal cachectic reaction.

Representative autopsy findings have been summarized in Table 2. In animals of group 3 given caffeine and fed laboratory chow, there was an increase in the absolute weight of adrenal and salivary glands and in parts of the gastrointestinal tissues. When laboratory chow was restricted in group 4, the same organs showed a slight gain or at least no loss in wet weight, which had markedly decreased in other body organs, and this was accompanied by a significant tendency to organ hydration. In groups 5 and 6 fed the rancid diet without caffeine, there was loss of absolute weight in most organs, particularly in muscle, skin, and thymus gland, but not including adrenals, brain, intestinal tissues and liver; this was accompanied by significant hydration of certain organs such as adrenals, parts of the gastrointestinal tract, and muscle. In rats of group 1 given caffeine and fed the rancid diet, there was a gain in the absolute weight of adrenals, salivary glands, parts of the gastrointestinal tract and liver and significant hydration of several organs as shown in Table 2. The results in groups 1 and 2 appeared to be a combination of the effects of caffeine, partial starvation and of feeding the rancid diet.

DISCUSSION

Feeding a cachexigenic rancid biotin-deficient diet increased the sensitivity of rats to the toxic effects of caffeine. Biotin deficiency was not involved. When the results were compared with those of feeding fresh biotin-deficient diet (Peters and Boyd, 1966a), rancidity may be seen to have partially protected the rats. When the same daily dose of caffeine was given to rats fed the fresh diet, the animals died earlier; controls fed the fresh diet without caffeine were similar to controls fed laboratory chow.

This paradoxical result may be due to the fact that animals fed the rancid diet ate less food during the first week than those fed the fresh diet. They therefore received less of those food factors which sensitized to caffeine toxicity or were exposed to a lesser degree of change in diet. Reduction of food intake is a relatively minor factor in caffeine toxicity (Peters, 1966).

The incidence of deaths due to automutilation after feeding rancid diet did not differ significantly from that after feeding fresh diet. But the convulsive type of death described by Boyd (1959) and seen after feeding the fresh diet (Peters and Boyd, 1966a) had disappeared and was replaced by a type of death which was similar to that following cachexia by the rancid diet alone. In this respect, the results agree with those obtained in measuring the effect of starvation on caffeine toxicity (Peters, 1966). In both studies caffeine apparently increased the need for calories in the starving animal or interfered with maximal utilization of the restricted food intake.

The rancid diet was not utilized as well as laboratory chow since calorie pair-fed animals did not lose as much body weight. Results in the pair-fed animals suggested that the early diuresis in rats fed the rancid diet was due to starvation and that starvation would account for some of the autopsy findings. The gain in liver weight (Table 2) appeared to be due to the rancid diet since it does not occur in rats fed the fresh diet (Peters and Krijnen, 1966). The presence of somewhat more liver tissue to metabolize caffeine in rats fed the rancid diet may have accounted for the initial partial resistance of these rats to the lethal effect of caffeine.

Autopsy results on animals which died or were moribund after caffeine administration and feeding of rancid diet and which had lost more weight than survivors showed an increase in organ water content of adrenal glands, gastrointestinal tract and muscle as was seen in animals dying from starvation alone (Peters and Boyd, 1966c).

The results show that anticipation of increased sensitivity to the toxic effects of drugs in malnourished animals need not necessarily be correct. The results for caffeine agree with increased barbiturate detoxification demonstrated in cachectic rats fed the same rancid diet (Peters and Boyd, 1966b).

SUMMARY

The effect of feeding a rancid cachexigenic diet upon caffeine toxicity was studied in albino rats. The diet alone produced loss of body weight, reduced food intake, diarrhea and an occasional death. Compared to feeding laboratory chow, the rancid diet increased caffeine toxicity and deaths due to cachexia. But compared to the same, but fresh diet, caffeine toxicity was relatively decreased during the first few days. These results show that malnutrition does not necessarily augment drug toxicity. Changes in clinical measurements and organ weights and water contents were found to be due to a combination of the effects of diet, caffeine administration and starvation.

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REFERENCES

BOYD, E. M. (1959). The acute oral toxicity of caffeine. *Toxicol. Appl. Pharmacol.* 1, 250–257.

BOYD, E. M., DOLMAN, M., KNIGHT, L. M., and SHEPPARD, E. P. (1965). The chronic oral toxicity of caffeine. *Can. J. Physiol. Pharmacol.* 43, 995–1007.

CROXTON, F. E. (1959). Elementary Statistics with Applications in Medicine and the Biological Sciences. Dover, New York.

PETERS, J. M. (1966). Caffeine toxicity in starved rats. *Toxicol. Appl. Pharmacol.* 9, 390–397.

PETERS, J. M., and BOYD, E. M. (1966a). Diet and caffeine toxicity in rats. *Toxicol. Appl. Pharmacol.* 8, 350–351.

PETERS, J. M., and BOYD, E. M. (1966b). Resistance to pentobarbital in rats fed a cachexigenic diet. *Toxicol. Appl. Pharmacol.* 8, 464–471.

PETERS, J. M., and BOYD, E. M. (1966c). Organ weights and water levels of the rat following reduced food intake. *J. Nutr.* 90, 354–360.

PETERS, J. M., and KAJNER, C. J. (1966). Organ weights and water contents of rats fed purified diets. *Growth* 30, 99–107.

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Caffeine Ulcerogenesis: A Mechanism Study in-Vivo[1]

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For a number of years the gastrointestinal ulcerogenic effect of caffeine has been recognized in cats (Judd, 1943; Roth and Ivy, 1944b) and in guinea pigs (Merendino *et al.*, 1945). However, exeriments involving other animals indicated a possible species difference in regard to this toxic effect of caffeine. Caffeine-induced ulcerogenesis in the dog has not been reported, and Roth and Ivy (1944a) confirmed the observation of Judd (1943) and other investigators that caffeine does not stimulate gastric secretion in this species. In contrast, early reports by Merendino *et al.* (1945), Musick *et al.* (1949), Grossman *et al.* (1945), and numerous other investigators indicate that caffeine stimulates acid and/or pepsin secretion in man and dogs. Since it has recently been reported (Pfeiffer and Gass, 1962) that gastric ulceration is induced by large doses of caffeine in the rat, the following experiments were conducted to elucidate the mechanism of caffeine induction of gastrointestinal ulceration.

METHODS

The gastric acid secretory effects of buffered solutions of caffeine alkaloid were investigated in the rat by the technique of Tsukamoto (1961), an assay based upon the intramural injection of drugs into the gastric submucosa. In addition, the influence of bilateral vagotomy and concurrent administration of parasympatholytic agents on caffeine-induced secretion was determined. This procedure allows assessment of the direct action of secretory stimulants or inhibitors on the gastric mucosa of anesthetized animals by visualization of topically applied, indicator-dye color changes.

In the present experiments, 201 healthy, adult, male and female rats were utilized. All were fasted at least 24 hours prior to surgery to ensure emptiness of the entire alimentary tract. The animals were lightly anesthetized with ether or sodium pentobarbital (35 mg/kg, i.p.), and all surgical and secretory studies were undertaken at 35–37°C in a surgical incubator. Test drugs were injected (0.05 ml/injection) intramurally into the submucosa of the glandular portion of exteriorized, everted stomachs. All test drugs were dissolved in buffered Ringer-Locke's solution (pH 7.3–7.4). Caffeine alkaloid was administered in doses of 0.001, 0.025, 0.50, and 1.0 mg. Control animals were injected and treated similarly with Ringer-Locke's solution.

In experiments to test for pharmacologic inhibition of caffeine-induced secretion

[1] A preliminary report was presented at the Forty-seventh Annual Meeting of the Federation of American Societies for Experimental Biology, Atlantic City, New Jersey, April 16-20, 1963. This work was supported by U. S. Public Health Service Research Grant No. A-5560, Division of Pharmacology and Experimental Therapeutics.

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atropine (0.05 mg or 5.00 mg) or scopolamine (0.05 mg) was injected intramurally at the site of secretion previously induced by intramural caffeine injection. Control animals received an injection of buffered Ringer-Locke's solution at the site of caffeine-induced secretion.

In all animals the latent period from the time of caffeine injection to the first appearance of gastric acid secretion, and the duration of secretion up to 1 hour were determined. Vagotomized rats were bilaterally and subdiaphragmatically vagotomized.

RESULTS

The influence of intramural caffeine on gastric acid secretion in the exteriorized rat stomach is indicated in Table 1. Sixteen out of twenty-one normal, intact rats (control

TABLE 1
CAFFEINE INDUCTION OF HYDROCHLORIC ACID SECRETION IN THE EXTERIORIZED RAT STOMACH

Dose of caffeine (mg)	Stomach preparation[a]	Response[b]	Latent period[c] (min)	Estimated mean duration[d] (min)
0.001	V	7/10	4.1 ± 1.1	22 (1)
	N	5/5	1.7 ± 0.8	19
0.025	V	9/10	3.8 ± 0.9	37 (4)
	N	10/10	0.8 ± 0.1	55 (9)
0.25	V	8/10	3.0 ± 0.4	15
	N	10/10	1.8 ± 0.2	18
0.50	V	8/10	2.1 ± 0.4	24
	N	10/10	1.5 ± 0.3	34
1.0	V	19/20	2.0 ± 0.3	28 (7)
	N	20/20	2.2 ± 0.3	26 (9)
Ringer-Locke (control)	V	1/15	9.5 ± 0.0	6
	N	16/21	1.4 ± 0.2	27 (5)

[a] "V" designates vagotomized rats; "N," normally innervated rats.
[b] Number of animals secreting/number dosed.
[c] Mean ± standard error.
[d] The number in parentheses is the number of rats secreting beyond 60 minutes. These animals are not included in calculation of the mean.

animals) secreted hydrochloric acid spontaneously under fasting conditions, whereas 14/15 of the bilaterally vagotomized rats did not secrete acid. This finding concurs with the reports of other investigators (Friedman, 1943; Komarov *et al.*, 1944), and illustrates that, under the conditions of the present experiment, accurate evidence of caffeine induction of gastric acid secretion can be obtained only from vagotomized animals. Submucosal injection of caffeine initiated gastric acid secretion in vagotomized rats. Although a dose-response relationship was not observed in regard to duration of secretion, it was observed in vagotomized rats that larger doses of caffeine initiated secretion more rapidly than smaller doses (shorter latent periods). In addition, only 7/10 of the vagotomized animals treated with 0.001 mg of caffeine secreted acid in contrast to 19/20 of the vagotomized rats treated with 1.0 mg caffeine; 80–90% of vagotomized animals treated with intermediate doses secreted hydrochloric acid. In general, normal rats secreted gastric acid more rapidly than vagotomized rats following caffeine stimulation; the shorter latent period is probably in-

dicative of spontaneous secretion rather than caffeine-induced secretion. In contrast to the vagotomized rats, which did not all respond to caffeine stimulation, all the normal rats demonstrated acid secretion following the injection of caffeine in any amount.

The results of the investigation of pharmacologic inhibition of caffeine-induced secretion are indicated in Table 2. Atropine and scopolamine were administered after

TABLE 2

INHIBITION OF CAFFEINE-INDUCED GASTRIC ACID SECRETION IN THE RAT

Treatment following 1 mg of caffeine	Stomach preparation[a]	Number dosed	Number secreting[b]	Number inhibited[c]
0.05 mg of atropine	V	10	10	10
5.0 mg of atropine	V	10	10	9
	N	10	10	10
0.05 mg of scopolamine	V	10	10	10
0.05 ml of Ringer-Locke	V	5	5	0
	N	5	5	0

[a] "V" designates vagotomized rats; "N," normally innervated rats.
[b] After administration of caffeine.
[c] By treatment in first column.

caffeine-induced secretion was well established, usually 10 minutes after the latent period. Doses of 0.05 and 5.0 mg of atropine, and 0.05 mg of scopolamine inhibited acid secretion in almost all cases. This inhibition was usually characterized by abolition, or occasionally by marked depression, of acid secretion. In control animals injected with Ringer-Locke's solution at the site of caffeine injection, secretion was not inhibited.

DISCUSSION

Gastric hypersecretion is frequently involved in the pathogenesis of gastric and duodenal ulcer. Thus, an evaluation of the possible stimulatory effect of caffeine on gastric acid secretion in the rat may help elucidate the etiology of caffeine-induced ulcerogenesis. The three parameters (percentage of animals stimulated, mean latent period, and mean duration) studied demonstrated that caffeine stimulates gastric acid secretion when administered submucosally.

Although the central neural influence and cephalic component of gastric secretion mediated via the vagus nerves can be eliminated by bilateral vagotomy, the peripheral neurons originating in the myenteric and submucous plexuses remain intact following this procedure. Thus, it can be hypothesized that the pharmacodynamic action of caffeine in vagotomized rats is limited to direct stimulation of the parietal cells, stimulation of the intramural, neural plexuses within the stomach, or to both of these mechanisms. Since it was demonstrated that the parasympatholytic agents atropine and scopolamine markedly inhibited the secretion, it can further be hypothesized that the stimulation induced by caffeine is primarily, if not entirely, mediated through the myenteric and submucous plexuses.

The acid-stimulating effect of caffeine has been observed by many investigators (Roth and Ivy, 1944a-c; Roth *et al.*, 1944; Grossman *et al.*, 1945; Merendino *et al.*, 1945; Musick *et al.*, 1946, 1949; Roth and Ivy, 1946). However, previous workers

(Ivy and Lane, 1956) were unable to find a dose of caffeine which, when administered subcutaneously, stimulated secretion in the rat. Roth and Ivy (1945) reported that caffeine-induced secretion in the cat was not abolished by bilateral vagotomy or subcutaneous atropine. They hypothesized that caffeine stimulates gastric secretion by acting peripherally on some mechanism in the gastric mucosa, probably directly on the parietal cells. The discrepancy between this and the present observation may be attributed to the differences in routes of administration. Roth and Ivy (1945) applied caffeine solutions directly to the gastric mucosa (gastric gavage) and superficial parietal cells, but administered the inhibitor subcutaneously, a factor which would result in slow absorption and consequent dilution. We administered a more dilute solution of caffeine to a site more remote from the parietal cells (gastric submucosa), and administered the inhibitor at a site less subject to problems of absorption, dilution, and circulation. Thus, the conditions utilized here facilitated the pharmacodynamic action of atropine in regard to gastric acid inhibition. In view of the results presented herein, as well as numerous other reports of caffeine-induced secretion and ulcerogenesis, it can be concluded that the primary mode of action of caffeine in ulcerogenesis in the rat is probably augmentation of gastric secretion. This enhancement of secretion is probably the result of both peripheral stimulation (including stimulation of the internal gastric nerve networks) and central stimulation following caffeine administration. In conjunction with the former mechanism, Schanker *et al.* (1957) has demonstrated that caffeine is absorbed in the rat stomach.

The observation that normal (nonvagotomized) rats secreted acid during fasting conditions independent of drug administration, concurs with the reports of Friedman (1943), Komarov *et al.* (1944), and Donald and Code (1952), who described the normal physiologic secretion of acid in fasting rats. Similarly, Brodie and Marshall (1963) reported that anesthetized primates normally secrete gastric acid under fasting conditions. In addition, the lack of gastric acid secretion in vagotomized control rats confirms the reports of Shay *et al.* (1949) and Lin and Alphin (1958) that they found no free acid in bilaterally vagotomized rats during fasting conditions.

SUMMARY

Gastric, submucosal administration of caffeine induced hydrochloric acid secretion in the rat. Evidence obtained from bilaterally vagotomized rats treated with parasympatholytic agents indicated that caffeine stimulation is mediated via intramural myenteric plexuses within the stomach. It is concluded that the primary mode of action of caffeine in ulcerogenesis in the rat is augmentation of gastric secretion.

The spontaneous secretion of gastric acid in fasting rats was confirmed.

REFERENCES

Brodie, D. A., and Marshall, R. W. (1963). Gastric contents of fasted primates: A survey. *Science* **141**, 174-175.

Donald, D. E., and Code, C. F. (1952). A study of gastric secretion in fasting rats. *Gastroenterology* **20**, 298-303.

Friedman, H. M. F. (1943). Histamine ineffective in the rat as a gastric secretory stimulant. *Proc. Soc. Exptl. Biol. Med.* **54**, 42-44.

Grossman, M. I., Roth, J. A., and Ivy, A. C. (1945). Pepsin secretion in response to caffeine *Gastroenterology* **4**, 251-256.

Ivy, A. C., and Lane, A. (1956). Effect of drugs on gastric secretion in the rat. *Am. J. Physiol.* **187**, 607.

JUDD, E. S., JR. (1943). Experimental production of peptic ulcers with caffein. *Bull. Am. Coll. Surg.* **28**, 46-47.

KOMAROV, S. A., SHAY, H., RAYPORT, M., and FELS, S. S. (1944). Some observations on gastric secretion in normal rats. *Gastroenterology* **3**, 406-413.

LIN, T. M., and ALPHIN, R. S. (1958). Cephalic phase of gastric secretion in the rat. *Am. J Physiol.* **192**, 23-26.

MERENDINO, K. A., JUDD, E. S., BARONOFSKY, I., LITOW, S. S., LANKIN, B. G., and WANGENSTEEN, O. H. (1945). Influence of caffeine on ulcer genesis. *Surgery* **17**, 650-666.

MUSICK, V. H., AVEY, H. T., HOPPS, H. C., and HELLBAUM, A. A. (1946). Gastric secretion in duodenal ulcer in remission—response to the caffeine test meal. *Gastroenterology* **7**, 332-339.

MUSICK, V. H., HOPPS, H. C., AVEY, H. T., and HELLBAUM, A. A. (1949). A simplified caffeine gastric test meal for the diagnosis of peptic ulcer. *J. Am. Med. Assoc.* **141**, 839-841.

PFEIFFER, C. J., and GASS, G. H. (1962). Caffeine-induced ulcerogenesis in the rat. *Can. J. Biochem Physiol.* **40**, 1473-1476.

ROTH, J. A., and IVY, A. C. (1944a). The effect of caffeine upon gastric secretion in the dog, cat and man. *Am. J. Physiol.* **141**, 454-461.

ROTH, J. A., and IVY, A. C. (1944b). The experimental production of acute and subacute gastric ulcers in cats by the intramuscular injection of caffeine in beeswax. *Gastroenterology* **2**, 274-284.

ROTH, J. A., and IVY, A. C. (1944c). The synergistic effect of caffeine upon histamine in relation to gastric secretion. *Am. J. Physiol.* **142**, 107-113.

ROTH, J. A., and IVY, A. C. (1945). The effect of vagotomy and atropine upon caffeine stimulation of gastric secretion *Gastroenterology* **5**, 129-134.

ROTH, J. A., and IVY, A. C. (1946). Caffeine and "peptic" ulcer. *Gastroenterology* **7**, 576-582.

ROTH, J. A., IVY, A. C., and ATKINSON, A. J. (1944). Caffeine and "peptic" ulcer; relation of caffeine and caffeine-containing beverages to the pathogenesis, diagnosis, and management of "peptic" ulcer. *J. Am. Med. Assoc.* **126**, 814-818.

SCHANKER, L. S., SHORE, P. A., BRODIE, B. B., and HOGBEN, C. A. (1957). Absorption of drugs from the stomach. I. The rat. *J. Pharmacol. Exptl. Therap.* **120**, 528-539.

SHAY, H., KOMAROV, S. A., and GRUENSTEIN, M. (1949). Effects of vagotomy in the rat. *Arch. Surg.* **59**, 210-226.

TSUKAMOTO, M. (1961). Effects of histamine, Compound 48/80, atropine, and Diamox, administered to gastric submucosa, on acid secretion in the rat. *Gastroenterology* **41**, 572-579.

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Toxicity of Caffeine, Theobromine and Theophylline.

By

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(Received May 6, 1953)

In previous investigations the authors (1948 to 1952) have studied the toxicity of a number of alkaloids to white rats and to microorganisms. With several of the alkaloids, it was found that there was little correlation between these toxicities.

The present study of the toxicity of caffeine, theobromine and theophylline to white rats and microorganisms resolved itself into determining: (A) the toxic effects of the alkaloids for white rats when injected intraperitoneally; (B) the effects of the alkaloids on the normal fermentative action of bacteria of the genus *Escherichia* and of the genus *Aerobacter*; (C) the germicidal and antiseptic value of the members of this group of alkaloids compared with phenol; and (D) from these results any correlation between the toxic action of the alkaloids for microorganisms and for white rats.

Animal Studies.

The methods and procedures were essentially the same as those followed in the investigations mentioned above and will not be discussed here in detail.

Because of rather slow action, the period of observing for record the animals injected with caffeine and theophylline was 36 hours.

Tables 1 and 2 record the intraperitoneal toxicity of caffeine and theophylline for white rats 1½ months old, equal numbers of male and female animals at each level injected. Table 3 shows results for rats of different ages. One group was from 2 to 4 months old and the other group from 6 to 9 months. Figure 1 shows the Characteristic Curves and the Dose-Mortality Curves, no attempt having been made to smooth them. The dose-mortality and characteristic curves for the alkaloids studied are found to approximate the *S*-type curves of BURN (1937) and TREVAN (1927) closely enough to indicate reliably uniform results.

[1]) Deceased.

Table 1.

Toxicity of Caffeine to White Rats, Age 1½ Months by Intraperitoneal Injection

Dose Alkaloid mg/kg	pH of Solution	Number Rats Injected	Number of Deaths	Per Cent Mortality	Av. Time to Death Hrs.	Av. Time to Death Mins.	Deaths after Convulsions	Survivors of Convulsions	Av. Time to first Convulsions (Minutes)
100	3.3	10	0	0					
150	3.3	10	3	30	19	59	1	0	8
200	3.3	16	8	50	16	25	6	2	4
210	3.2	20	15	75	11	51	9	0	4
225	3.4	20	18	90	15	55	13	0	4
230	3.2	10	10	100	11	15	9	0	4
275	3.2	13	13	100	2	48	7	0	3

Table 2.

Toxicity of Theophylline to White Rats, Age 1½ Months, by Intraperitoneal Injection.

Dose Alkaloid mg/kg	pH of Solution	Number Rats Injected	Number of Deaths	Per cent Mortality	Av. Time to Death Hrs.	Av. Time to Death Mins.	Deaths after Convulsion	Survivors of Convulsions	Av. Time to first Convulsions (Minutes)
150	5.5	10	0	0					
175	5.6	15	1	7	12	5	0		
200	5.6	15	4	27	11	15	1	0	55
210	5.6	30	19	63	4	16	8	0	42
215	5.6	80	64	80	3	30	36	0	33
225	5.6	20	19	95	3	10	8	0	37
250	5.5	10	10	100	1	49	6	0	23
275	5.6	10	10	100	1	32	4	0	20

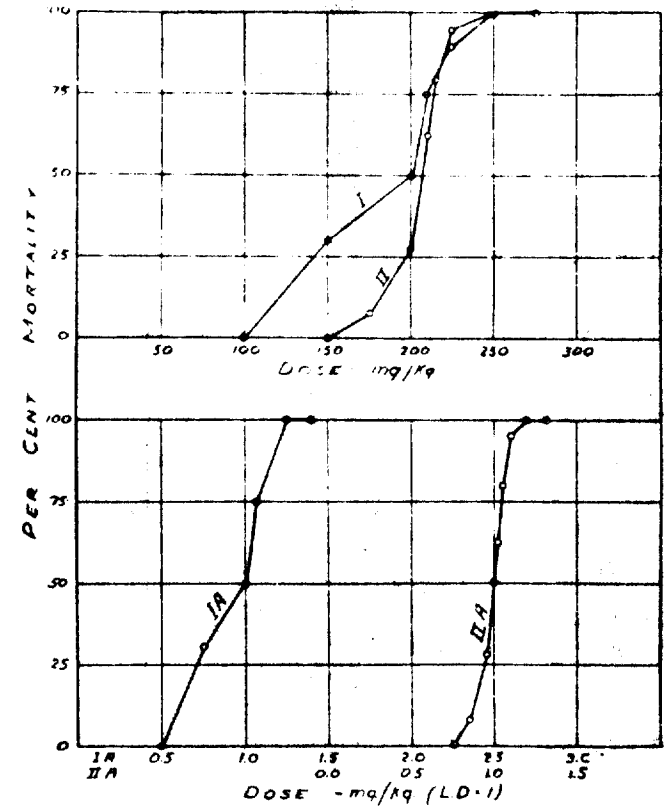

Fig. 1. Dose-Mortality Curves for I Caffeine and II Theophylline. Characteristic Curves for IA Caffeine and IIA Theophylline.

The LD 50 interpolated on the dose-mortality curves for each of the different alkaloids is as follows: caffeine, 200 mg/kg for animals 1½ month old, 205 mg/kg for animals from 2 to 4 months and 167 mg/kg for old rats; and for theophylline, 206 mg/kg for animals 1½ months old.

The degree of toxicity found for caffeine compares favorably with the results reported by SALANT and RIEGER (1912) for intraperitoneal injections into rabbits, guinea pigs, and cats. For these animals the M.F.D. (minimum fatal-dose) varied from 150 to 250 mg/kg. KISSKALT (1915) reported LD 50 to be approximately 260 mg/kg for white rats.

The toxic effects of caffeine were evidenced first by nervous movements of the animals around the cage. The average time to the first convulsion was approximately 4 minutes. The convulsions were of a tetanic nature. 67% of all rats under continuous observation until death had tetanic convulsions preceding death, but all convulsions did not terminate in

Table 3.

Comparative Toxicity to white Rats of Different Ages of Caffeine by Intraperitoneal Injection.

Dose Alkaloid mg/kg	Young Rats (2 to 4 Months)			Old Rats		
	Number injected	Number Deaths	Per cent Mortality	Number injected	Number Deaths	Per cent Mortality
100	5	0	0	5	0	0
150	8	2	25	10	4	40
175				20	11	55
200	10	4	40	16	12	75
210	10	6	60	10	9	90
225	10	8	80	10	10	100
250	10	10	100	6	6	100
300	13	13	100			

death. The different age groups were about equally susceptible to caffeine. On a limited number of animals, the LD 50 was smaller for old rats.

Lethal doses of theophylline were generally followed by ten to fifteen minutes of lethargic inactivity, succeeded by nervous movements and finally by violent racing around the cage with tetanic spasms, after which time the animal died gasping for breath and with the four legs distended very rigidly. The convulsions for animals given this alkaloid were delayed much longer than for caffeine. The longest time to the first convulsion was 2 hours and 51 minutes, whereas the longest time to a convulsion with caffeine was 10 minutes. 50% of the deaths were not preceded by convulsions, but every animal having a convulsion died.

Bacteriological Studies.

Different amounts of the alkaloids were added to lactose phenol red broth, and the tubes were sterilized. Because of the low solubility of some of the alkaloids, a few of the lower dilutions contained some of the compounds as suspensions. Tubes of each different lot of media were inoculated separately with cultures of *Aerobacter* and *Escherichia*. The organisms used for seeding were from stock cultures collected, purified and standardized by careful selection in this laboratory over a number of years [illegible] Poe (1931). After inoculation, the tubes were incubated at 37° C. for 72 [illegible] Readings of gas production were made at 24, 48, and 72 hours [illegible] showed no gas in 72 hours the incubation was continued for 2, 4,

or even 7 additional days. The acidity of the medium, as indicated by the colour of the indicator, was recorded with the third reading. In practically all tubes in which growth was observed, the colour change in the indicator occurred at the end of 24 hours. Controls were inoculated for each determination.

Table 4 lists the average gas production during 72 hours obtained with 7 species of *Escherichia* and 3 species of *Aerobacter* in the presence of various concentrations of the 3 alkaloids.

The names of the organisms are listed below, with the designated numbers assigned to each.

Number	*Name of Organism*
29; 389	*E. coli*
62; 213; 217	*E. communior*
179; 404	*E. paragruenthali*
139; 345	*A. aerogenes*
190; 201	*A. oxytocum*
126; 148	*A. cloacae*

The values recorded in Table 4 were obtained by dividing the volumes of gas in percentages produced at the end of 24, 48, and 72 hours in tubes containing the various dilutions of alkaloids by those produced in the same times by the lactose broth controls of the same organisms. A typical reading showed that organism number 179 produced 10, 18, and 36% of gas at the end of 24, 48, and 72 hours, respectively, in a dilution of caffeine = 3.6 mg/ml. The corresponding readings for the controls were 25, 30, and 40%. The average relative readings recorded for this concentration of caffeine were, therefore, 40, 60, and 90% of normal production. In terms of gas production, a value of 40 opposite a certain concentration of an alkaloid means that the organism is 40% normal in its gas production in the particular medium. The results were thus recorded so as the more easily to observe the extent to which the alkaloid had affected the normal functioning of the organism.

In Table 5 are recorded the effects of the different alkaloids on the growth of the individual species of the *Escherichia* and *Aerobacter* genera.

Caffeine and theophylline were about equally toxic for the members of each genus of bacteria and are comparatively much more toxic than theobromine. In media containing as much as 67 mg per ml of theobromine, growth of all the organisms took place. Little difference in resistance was shown by the individual members of the two genera of bacteria in media made with any given alkaloid. There were a few instances where the alkaloids actually stimulated a greater gas production than that produced in the lactose controls. This condition was especially noticeable with species of the genus *Aerobacter*. With the highest concentration 67 mg/ml of the theobromine used there was a decided stimulation of

Table 4.

Average Gas Production in Alkaloidal Media, with Broth Controls Taken as 100

Alkaloid	Concentration of Alkaloid as Base in mg per ml	*Escherichia* Hours			*Aerobacter* Hours		
		24	48	72	24	48	72
Caffeine	0.1	90	95	95	62	74	86
	0.2	80	85	90	50	90	105
	1.0	66	86	90	43	87	107
	2.0	17	48	62	20	100	108
	2.5	24	61	70	5	52	75
	3.3	5	12	20	0	10	30
	3.6	4	10	24	0	18	31
	4.0	0	5	5	0	0	8
	5.0	0	0	0	0	0	0
Theobromine	0.1	79	89	89	132	120	105
	0.2	90	100	105	120	128	110
	1.0	118	104	108	105	121	118
	10.0	78	89	89	69	93	99
	12.5	74	74	74	73	68	72
	20.0	59	60	60	70	99	95
	33.0	52	56	58	93	79	78
	66.7	108	103	100	127	114	110
Theophylline	0.1	72	93	93	118	106	106
	0.2	70	82	90	100	110	105
	1.0	67	75	82	78	100	101
	3.3	7	11	18	0	5	18
	4.0	0	0	10	0	0	17
	5.0	0	0	0	0	0	0

gas production over the lactose controls. Delayed fermentation was noticed in some of the media containing caffeine and theophylline. The stimulation, however, with the lower concentrations of caffeine was not so marked as reported by MEZZEDROLI and SGARZI (1936), AMATI and SGARZI (1925), SECHI (1930), and REED and WILLIAMS (1915). HAIM and TORRES (1927) stated that ROTH and KLOUMANN found *B. coli* slightly affected by large concentrations of caffeine, whereas *B. typhi* was not affected at all.

The next series of experiments was designed to test the *germicidal activity* of the alkaloids compared to phenol. The United States Department of Agriculture method, RUEHLE and BREWER (1931), for testing germicides and antiseptics was used first. The alkaloid media were adjusted to a pH as near 4 as possible.

In nutrient broth medium saturated with the salts of the alkaloids,

Table 5.

Effect of Alkaloids on the Growth of Individual Cultures of *Escherichia* and *Aerobacter*

Concentration of Alkaloid as Base in mg per ml	*Escherichia* Culture Number							*Aerobacter* Culture Number					
	62	213	217	29	389	179	404	201	196	148	126	345	139
Caffeine													
2.0	—	—	—	—	—	—	—	—	—	—	—	—	—
2.5	—	—	—	—	—	—	—	—	—	—	—	—	—
3.3	—	—	—	—	—	—	—	—	+	+	—	—	—
3.6	—	—	—	—	—	—	—	—	+	—	—	—	*
4.0	—	—	*	—	*	—	—	*	—	—	—	—	—
5.0	—	—	—	—	—	—	—	—	—	—	—	—	—
Theobromine													
10.0	—	—	—	—	—	—	—	—	—	+	+	—	—
12.5	—	—	—	—	—	—	—	—	—	—	—	—	+
20.0	+	—	—	—	—	—	—	—	—	—	+	+	—
33.0	+	—	—	—	—	—	—	—	—	—	+	+	+
66.7	+	+	—	—	—	—	—	—	+	—	+	+	+
Theophylline													
1.0	+	+	+	+	—	—	—	—	—	+	+	+	+
2.0	+	+	+	+	—	—	—	—	—	—	+	+	+
3.3	+	+	—	+	—	—*	—	+	+*	+	+	+	+
4.0	+*	—	—	+	+	—	—	—*	—	—	+	—	+
5.0	—	—	—	—	—	—	—	—	—	—	—	—	—
6.7	—	—	—	—	—	—	—	—	—	—	—	—	—

\+ Indicates growth and gas production
* Indicates that a period of more than 72 hours was required for gas production.

growth of both *E. typhi* and *S. aureus* took place in the presence of all three alkaloids after 15 minutes exposure to them in the media.

With the wet filter paper method, very little effect was shown by the alkaloids with *S. aureus* as the test organism, growth being inhibited in saturated solutions of the salts of the alkaloids after the test organism was exposed to them for the following periods of time: caffeine, 50 minutes; theobromine, 15 minutes; and theophylline, 15 minutes.

Summary.

1. The toxicities of caffeine, theobromine, and theophylline have been determined.

2. Caffeine and theophylline are much more toxic for bacteria than theobromine.

3. Caffeine and theophylline show about the same toxicity for white rats.

4. Caffeine is slightly more toxic for old rats than for the young animals.

REFERENCES

Amati, A., and L. Sgarzi: *Giorn. biol. ind. agrar. aliment.* 1925, **5**, 52; See. *C. A.* 1936, **30**, 6125.

Burn, J. M.: *Biological Standardization.* Oxford University Press. London, 1937.

Drummond, F. G., C. C. Johnson, and C. F. Poe: *Acta pharmacol. et toxicol.* 1950, **6**, 235.

Hann, Arthur, and Torres: *Bakt. Parasitenk. I Abt., Orig.* 1927, **102**, 96.

Johnson, C. C., Gladys Johnson, and C. F. Poe: *Acta pharmacol. et toxicol.* 1949, **5**, 222.

Johnson, C. C., Gladys Johnson, and C. F. Poe: *Acta pharmacol. et toxicol.* 1952, **8**, 71.

Johnson, C. C., and C. F. Poe: *Acta pharmacol. et toxicol.* 1948, **4**, 265.

Kisskalt, Karl: *Biochem. Zeitschr.* 1915, **71**, 470.

Mezzadroli, G. and L. Sgarzi: *Giorn. biol. appl. ind. chim.* 1935, **4**, 162. See. *C. A.* 1936, **30**, 8470.

Poe, C. F.: *J. Amer. Water Works Assoc.* 1931, **23**, 1218.

Poe, C. F., and C. C. Johnson: *Acta pharmacol. et toxicol.* 1949, **5**, 110.

Poe, C. F., C. C. Johnson, and Gladys Johnson: *Univ. of Colo. Studies, Series in Chem. and Phar.*, 1952, **52**, 65.

Reed, H. S. and B. Williams: *Zentr. Bakt. Parasitenk.* 1915, **II**, **43**, 166.

Ruehle, G. L. A. and C. M. Brewer: *U. S. Dept. of Agric.* Circular 198, 1931.

Salant, W., and J. B. Rieger: *U. S. Dept. of Agric., Chem. Bureau Bull.* No. 148, 1912.

Sechi, E.: *Arch. intern. pharmacodynamie*, 1930, **37**, 181.

Trevan, J. W.: *Proc. Roy. Soc.* (London), B. 1927, **101**, 483.

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Facts about Caffeine, Theobromine in Foods

By P. W. PUNNETT
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Effects of caffeine on the human system have heretofore been unduly criticized, and a bad reputation has resulted for some food products containing it. This article tells you the facts about a few of the foods and beverages containing caffeine and its "cousin" theobromine. Considerable quantities are consumed without any bad effects

PREJUDICE against the use of beverages which contain caffeine is common. It has as its basis complete ignorance of certain facts, namely, the physiological action of caffeine and the occurrence or presence of caffeine in a number of common foods and beverages.

Probably the term "stimulant" has an unfortunate connotation for most people. It suggests "alcohol" or alcoholic beverages, drunkenness, hangovers, physical deterioration and other objectional phenomena attending its abuse. This is, of course, a fallacy in itself for alcohol is essentially a depressant and not a stimulant, particularly when it is consumed too often or in too large quantities.

The action of caffeine on the human system is very different from that of alcohol, as will be found by consulting pharmacological and physiological authorities. The literature is voluminous and would require many pages for a review of all the work which has been done on its physiological and psychological effects. But it has been termed "the stimulant without a hangover"—when its effect wears off the body returns to its ordinary level instead of sagging below to a state of depression.

The other cause of the prejudice is the lack of appreciation of the fact that caffeine is present in a number of other food products besides coffee. Coffee has been the "goat"—the horrible bugaboo that is held up to scorn in our schools and in our magazines and newspapers. How this idea began no one can say for certain. But it is one of the many misconceptions that "everybody knows."

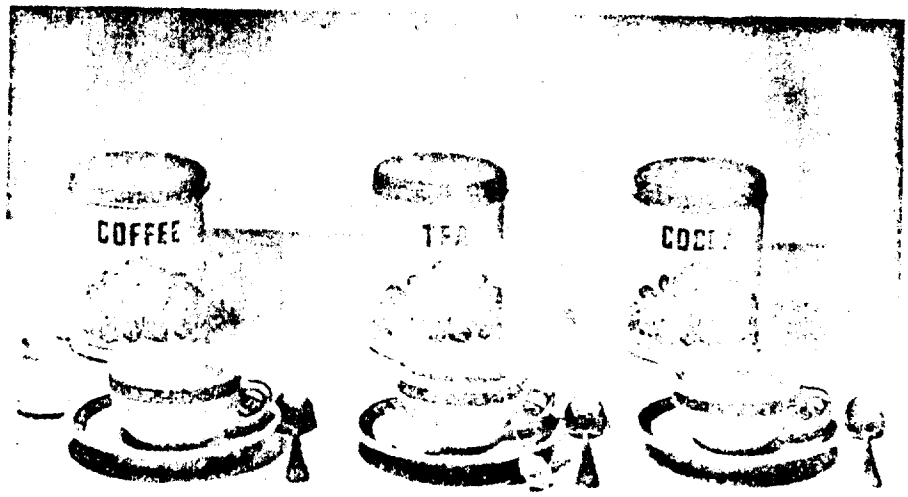
Not only is caffeine considered a dangerous substance found only in coffee—in the popular mind—but there is a complete lack of knowledge regarding another closely allied substance, theobromine. This is present in considerable amounts (along with more or less caffeine) in all chocolate products. It is chemically a first cousin of caffeine and, in fact, is sometimes converted into caffeine for commercial purposes. It has a similar stimulating action, though probably to a less degree. Medically both are used as stimulants, even in cases of heart ailments.

In order to lend point to the above remarks and to provide up-to-date useful data on the presence of caffeine and theobromine in various ordinary foods, a number of analyses have been made and the results summarized in Table I.

Caffeine in Tea and Coffee

In determining caffeine in tea and coffee, the analysis was performed on brews by several common methods. The tea brew was prepared as follows:

A. 900 c.c. (almost 1 qt.) of water at a temperature of 90 deg. C. (194 deg. F.) was poured on 13.5 grams of loose tea. The temperature was kept at 90 deg. C. for 5 minutes and then the brew poured off through a strainer. The



Analysis shows that for the average rate of consumption of food and beverages, such as coffee, tea and cocoa, the caffeine and theobromine content are not enough to cause any bad effects on consumers.

tea and water used are in proportion of 1 oz. of tea to 2 qt. of water.

B. 900 c.c. of water at 90 deg. C. was poured over six tea bags. The contents of a bag had previously been found to average 2.28 grams, and hence the six bags used contained 13.68 grams of tea. The brewing was held at 90 deg. C. for 5 minutes and the bags then removed.

Coffee brew for the caffeine determinations was prepared in the following manner:

A. Drip method—950 c.c. of boiling water was poured into the upper compartment of a stainless steel drip pot containing 48 grams of coffee. This proportion is two level tablespoonfuls per ½ pt. of water. This is a commonly accepted, average recipe. A fairly fine or "drip" grind of coffee was used.

B. Percolator method—The same proportion of coffee (in a regular or percolator grind) and water were used as in (*A*). The brewing was continued for 10 minutes after the water being pumped up showed a barely perceptible tinge of color.

C. Vacuum coffee maker method—The same proportions of coffee (ground very fine—pulverized but not floury) and water used as in (*A*). This was placed in a glass vacuum-type coffee maker on an electric heater. As soon as all the water possible has been driven into the upper bowl, the coffee-water mixture was stirred and the heat turned off, allowing the brew to filter into the lower bowl.

In addition to the above types of food products caffeine determinations were made in duplicate on two well known carbonated beverages of the "cola" type. In determining the caffeine in the tea and coffee brews and in the carbonated beverages, the Power-Chestnut Method[1] was used, with the exception that a measured portion of the beverage was taken instead of the alcoholic extract from 10 grams of coffee. To this was added the 10 grams of magnesium oxide, the mixture evaporated and the determinations made according to the Power-Chestnut Method.

Caffeine and Theobromine in Chocolate Products

Both caffeine and theobromine were determined on well-known brands of breakfast cocoa, baking chocolate and sweet milk chocolate (sold in the form of bars as a confection).

Cocoa—Directions on the package suggested the use of 1 heaping teaspoonful (average 4.5 grams) per 8 oz. of milk and water. This should make one and a half servings of the beverage, and hence the caffeine and theobromine content were calculated accordingly. The method of analysis was that of Decker-Kunze,[2] with the exception that instead of filtering the water extracts obtained by boiling the sample with magnesium oxide and water, they were centrifuged and the decanted liquid poured through filter paper. This was found to be far more rapid than suction filtration.

Baking Chocolate—Directions on the wrapper of baking chocolate suggested the use of 2 oz. (56.7 grams) to 1 qt. of milk and water. This would make six servings and the results were calculated accordingly. The same method (Decker-Kunze) was used.

TABLE I—Amounts of Caffeine and Theobromine in Various Foods.

	Caffeine (mg.)	Theobromine (mg.)
Tea (per 5-fl. oz. cup)		
Brand *A*, loose	75.6	.....
Brand *A*, tea bags	62.9	.....
Brand *B*, loose	65.4	.....
Brand *C*, loose	60.2	.....
Coffee (per 5-fl. oz. cup)		
Brand *A*, drip method	67.8	.....
Brand *A*, percolator method	100.9	.....
Brand *A*, vacuum maker method	88.9	.....
Brand *B*, drip method	76.9	.....
Brand *C*, drip method	97.3	.....
"Cola" type beverage (per bottle)		
Brand *A*, 6 oz.	37.2	.....
Brand *A*, 6 oz.	38.3	.....
Brand *B*, 12 oz.	112.4	.....
Brand *B*, 12 oz.	100.8	.....
Cacao products		
Breakfast cocoa (5-oz. cup)	1.1	55.1
Baking chocolate (5-oz. cup)	7.8	125.7
Sweet milk chocolate (10 cent bar)		
Chloroform extraction	58.1	301.4
Tetrachloroethane extraction	78.0	439.0

TABLE II—Approximate Solubility of Theobromine.

Solvents	Mg. per 100 c.c.
Benzene	5.0
Carbon tetrachloride	5.0
Ethyl acetate	11.0
Petroleum ether	10.0
Ethyl ether	25.0
Trichloroethylene	193.0
Ethylene dichloride	0
Dichloroethylene	88.0
Ethylidene chloride	40.0
n-Butyl chloride	14.0
Methyl chloroform	0
Iso-propyl ether	12.0
Iso-propyl acetate	10.0
Iso-propyl chloride	9.0
Carbon bisulfide	2.0

Sweet Milk Chocolate—This product is, of course, eaten "as is." A 10-cent bar was found to weigh 98.5 grams (weight stated 3½ oz. or 99.7 grams). On attempting to use the Decker-Kunze method, certain fundamental difficulties arose that made changes absolutely necessary.

The first was a physical difficulty. In the Decker-Kunze method, the hot water extract obtained as mentioned above is supposed to be evaporated to dryness on sand. This mass is put into an extraction thimble and extracted for eight hours or more with hot chloroform in a continuous fat extractor. This is entirely satisfactory in the case of cocoa or chocolate, but when considerable sugar is present, as is the case with sweetened chocolates, the sugar extracted by the hot water is a sticky mass when dried on the sand. This is almost impossible to dry thoroughly and almost impervious to the choloroform. Three attempts were made to perform the determinations by this process, using various proportions of sand and methods of drying. After several days of extracting only a fraction of the caffeine and theobromine was removed.

The second difficulty was a minor one. In the Decker-Kunze method, the dry mixture of caffeine and theobromine is treated with benzene to dissolve out the caffeine, theobromine being practically insoluble in this solvent. The theobromine is then determined by Kunze's method with excess silver nitrate, filtration and subsequent titration. This is satisfactory apparently in the case of pure cocoa and chocolate which contain little, if any, reducing sugars. In the case of sweetened chocolate, however, the chloroform extraction process or any other solvent extraction, as was determined by several experiments on water extracts of sweet chocolate, is also very apt to remove some sugar. This readily reacts with ammonial silver nitrate during the boiling of the theobromine residue. The answer to this difficulty was, of course, to determine nitrogen in the residue by Kjeldahl digestion and to calculate from it the amount of theobromine. It might be said here that all figures given in the table for caffeine and theobromine are based on nitrogen determinations and not direct weighings.

Theobromine is somewhat remarkable for its comparative insolubility in organic solvents. A number of approximate determinations of its solubility in various solvents at room temperature is shown in Table II.

Extraction Methods

Other investigators[3] have used a phenol-chloroform mixture for theobromine extractions, but it is stated elsewhere[4] that phenol-chloroform may extract other substances also. Harral[5] has found it easier to convert theobromine to caffeine and then extract. This is of course out of the question when the separate figures are desired. Wadsworth[6] and others[6,7] have used tetrachloroethane as an extractant.

It seemed worth while to try using

(*Turn to page 95*)

Facts About Caffeine

(Continued from page 53)

chloroethylene and tetrachloroethane solvents for the extraction of caffeine and theobromine from a water solution as may be obtained by applying first step of the Decker-Kunze method. The former, trichloroethylene, seemed preferable since its boiling point (87.9 deg. C.) is low enough to facilitate its removal by evaporation on a steam bath. It was found, however, that (in the supply available, at any rate) it could not be kept neutral even though it was washed free of acid and redistilled at intervals. When an attempt was made to use it in a continuous extractor at the Palkin type[5] the acidity increased so rapidly as to cause the separation of tarry materials from the solution. Use in a separatory funnel on several trials gave the same sort of results.

For the above reason, the use of the tetrachloroethane was considered. Its boiling point (144-146 deg. C.) is fairly high and there was a possibility that caffeine or theobromine, or both, might be lost if a solution of these substances in it was distilled down to small volumes (to recover most of the tetrachloroethane) and the balance evaporated on the steam bath with the aid of an air jet. Weighed amounts of caffeine U.S.P. and theobromine U.S.P. (purity determined in each case by nitrogen determinations) were added to 300 c.c. of tetrachloroethane, the solvents distilled off down to about 10 c.c. and the balance evaporated on the steam bath. The residue was treated with benzene, theobromine filtered from the solution of caffeine, and nitrogen determined on both. Recovery was 99.8 percent in each case. It was found to be impossible to use tetrachloroethane in a Palkin extractor since the high temperature of the vapor caused the water solution of the caffeine and theobromine to boil. It might be possible to modify this apparatus so that the hot vapor of the solvent would go directly to the condenser without passing through the extraction tube. If this were done, it undoubtedly would result in a saving of time and attention needed.

In the absence of such a device, trials were made of hand extraction in a separatory funnel. Weighed amounts of caffeine and theobromine were dissolved in 50 c.c. of warm water, cooled, washed into a separatory funnel to 75 c.c. and made faintly alkaline with sodium hydroxide. Four extractions, using 50 c.c. of tetrachloroethane each time, were made and combined in a round bottomed 500 c.c. flask. The solvent was then distilled off down to 10 c.c. This residue was extracted from the residue with benzene and nitrogen determinations made. All of the caffeine and about 75 percent of the theobromine were extracted by the first four extractions. Two more sets of four extractions each made in the same manner removed all of the theobromine.

The method was then applied to the sweetened milk chocolate mentioned above using a 15-gram sample which was boiled with magnesia and water, centrifuged and filtered as described above. The combined extracts were evaporated down to 50 c.c., washed into the separatory funnel to 75 c.c., made faintly alkaline and extracted with tetrachloroethane as above. The mixture tended to emulsify somewhat during the first few extractions but afterwards separated freely. The extracts were filtered through a small plug of cotton into the distilling flask in each case. All of the caffeine was extracted by 12 portions of solvent while the theobromine required 16 to 20 portions. This, of course, is a rather large number of extractions to have to use.

A sample of another bar of the same brand of chocolate was also analyzed by extracting with magnesia and hot water as above, evaporated to about 50 c.c. and then washed into the Palkin extractor. Chloroform was used in a continuous extraction for about 20 days (eight hours per day) until examination of the extract showed only minute amounts of theobromine being removed. Results by this method are also given in Table I.

Conclusions

It will be seen from Table I that in the case of tea 60 to 75 mg. (1¼ grains) of caffeine will be found in the average cup. The brewing time was five minutes. This is the time suggested by the tea industry and by tea lovers. Many may not use as long a brewing time as this, but other research has shown that 50 percent of the caffeine is extracted in the first minute of brewing, and practically all by the end of four minutes.

In the case of coffee, the amount of caffeine in the cup varied from 68 to 101 mg. (1 to 1½ grains), depending probably largely on the grind and the method used. Caffeine is extracted somewhat more slowly from coffee than from tea, and the time during which the coffee is in contact with the water will determine the amount in the cup. Unless excessive amounts of coffee are used, a cup of the brew probably will never contain much more than 100

mg. (this amounts to 1½ grains).

The two "cola" beverages contained different percentages of caffeine. Brand A contained 6½ mg. per fluid ounce, and B about 9 mg. As a rule one consumes a bottle at a time and in one case would get about 5.8 grains and in the other 1¾ grains of caffeine.

In the case of the cacao products, amounts of caffeine and theobromine vary widely. Apparently the removal of the fat from chocolate in the manufacture of cocoa results also in the removal of some of the caffeine and theobromine. At any rate in the cups of cocoa and chocolate the caffeine content is small. The theobromine content is over ¾ grain in the former and about 2 grains in the latter and its physiological action must be appreciable. In the case of bar milk chocolate, however, the caffeine consumed is about as much as in a cup of tea or coffee while the theobromine is 5 to 7 grains. These figures may seem high compared to some in the literature. This may be due to the fact that older methods did not really get out all of these substances, as well as to variations in the cacao used in the bar.

The list of products analyzed includes only a few of such beverages and foods which are commonly consumed very day both by adults and by children. There are many other "cola" type beverages on the market, some nationally distributed, some sectionally. There are many varieties and brands of chocolate and cocoa sold, and cacao products find their way into a variety of foods, such as ice creams, candies, chocolate-milk drinks, cakes, icings and desserts. When the list of such foods is considered, it is evident that practically every person consumes caffeine and theobromine containing foods and beverages very frequently and consumes appreciable quantities of caffeine and theobromine *with no noticeable bad effects*. It would seem time that the true facts are appreciated and efforts made to present them to the public in an unbiased form.

References

1. "Methods of Analysis of the A.O.A.C.", 5th Edition, p. 195, McGraw-Hill Book Co., New York, N. Y.
2. Leach and Winton, "Food Inspection and Analysis," 4th Edition, p. 413, John Wiley & Sons, New York, N. Y.
3. Parkes and Parkes, *Analyst*, vol. 62, 791, 1937.
4. Wadsworth, *Analyst*, vol. 46, 32, 1921.
5. Harral, *Analyst*, vol. 65, 408, 1940.
6. McDonald, Annual Report on Cacao Research, Imperial College of Tropical Agriculture, Trinidad, 1936.
7. Humphries, Annual Report on Cacao Research, Imperial College of Tropical Agriculture, Trinidad, 1938-39.
8. Palkin, Murray and Watkins, *Industrial and Engineering Chemistry*, vol. 17, 612, 1925.

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202(12):1105-1106. 1967.

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Caffeinism

A Cause of Long-Continued, Low-Grade Fever

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Low-grade irregular fever, insomnia, anorexia, and irritability affected a psychoneurotic woman for months. She habitually drank an excessive amount of coffee. Fever and the symptoms disappeared after the intake of coffee was reduced. Fever is one of the manifestations of caffeinism.

THE EXCESSIVE USE of common household items including sedative drugs, cigarettes, alcohol, tea, coffee, dentifrices,[1] and even peanuts[2] can induce illness mistaken for other disease. The cause is easily overlooked unless a patient's habits are discovered. Prompt recognition obviates much clinical effort, laboratory testing, unnecessary therapy, and expense. Removal of the cause is the cure.

Report of a Case

A housewife, aged 39 years, complained of having a "cold" for six months because of almost daily low-grade fever with occasional flushing and chilliness, insomnia, irritability, anorexia, loss of 20 lb (9.1 kg) of weight, and conjunctival irritation. Cramps in the epigastrium and of the toes and cold hands and feet occurred at times. The oral temperature in the daytime usually rose to 99 F (37.2 C) or more, and occasionally to 100.1 F (38 C). Phenobarbital was taken nightly to induce sleep. Five abdominal operations had been done during 20 years, and menses ceased after total hysterectomy in 1963. She has two healthy children. Three genetic relatives have diabetes.

In June 1967, after detecting leukocytes and albumin in a sample of urine, probably not collected with proper care, her physician suspected pyelonephritis, possibly as a result of ureteral obstruction from scar formation after the numerous surgical procedures. The patient never had dysuria. No other abnormalities were found on physical examination nor in laboratory studies. Because antimicrobic therapy failed to influence the temperature and the symptoms, the patient was sent to the hospital for investigation.

On admission at 4 PM, her oral temperature was 99.4 F (37.5 C); pulse rate, 100 beats per minute; respiration rate, 18/min; and the blood pressure, 120/80 mm Hg. She was apprehensive, pallid, and weighed 107 lb (48.5 kg). No abnormalities were evident on physical examination. The extremities were cool and dry, the reflexes were normal, and there was no tremor. Conditions for diagnostic consideration were, among others, pyelonephritis, diabetes, hyperthyroidism, tuberculosis, lymphoma, psychoneurosis, habitual hyperthermia, and factitious fever.

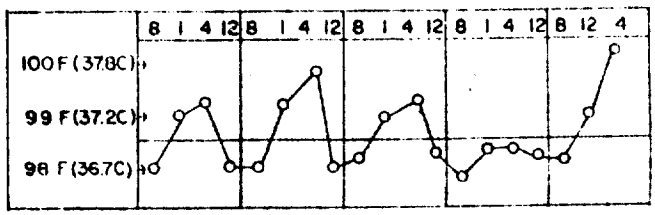
During five days in the hospital, no fever occurred. Rectal temperature averaged 98.8 F (37.1 C); the highest temperature on two days was 99.8 F (37.7 C); the lowest, 97 F (36.1 C). Oral temperature was 0.5 to 1 F degree lower. The pulse rate fluctuated between 70 and 100 beats per minute, averaging 80 per minute. The blood pressure declined to 105/60 mm Hg.

Daily urinalyses disclosed no abnormality, except on one occasion a trace of albumin and a few diphtheroid bacilli were found. Urine cultures remained sterile. An intravenous pyelogram showed no lesion in the kidneys, ureters, or bladder. Two fasting-blood-sugar determinations revealed 90 mg/100 ml and 102 mg/100 ml. Two two-hour samples of blood contained 251 mg and 173 mg of glucose, respectively, but glycosuria was absent in ten samples of urine tested during three days. A roentgenogram disclosed no pulmonic infiltration, and dermal tests with tuberculin, histoplasmin, and coccidioidin were nonreactive. The hemoglobin value was 13.8 gm/100 cc; the leukocytes numbered 8,800/cu mm with normal constituent cells.

Thus far, none of the data gave clues to the cause of fever, loss of weight, insomnia, and albuminuria. The patient has a compulsive personality but denied that social, economic, or other factors would account for emotional disturbance. Habitual hyperthermia or factitious fever are not accompanied by loss of weight or insomnia.[1] Normal hyperthermia related to the menstrual cycle was not likely after menses ceased in 1963.[1]

On further inquiry, the patient stated that she worked as a waitress from 5 PM to 11 PM. During the day she smoked a pack or more of cigarettes and drank from 15 to 18 cups of brewed coffee from 8 AM until 4 PM when she left home for work. Oral temperature, measured at times by the patient's physician, at times by me, and four times a day by her, usually exceeded the norm and occasionally rose to 100.4 F (38 C) only between noon and late afternoon, as shown in the Figure. This behavior, the normal temperature observed in the hospital where only one cup of coffee a day was served, and the symptoms

Oral temperature recorded by patient at 8 AM, 1, 4, and 12 PM.



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suggested caffeinism. The response to caffeine begins from 30 to 60 minutes after ingestion[5,6] and persists for several hours, which is consistent with the patient's daily timed appearance and disappearance of fever.

The patient had no withdrawal symptoms after the intake of coffee was restricted. Usually, headache and lassitude ensue a few hours after deprivation of the accustomed amount in habituated persons.[7] The temperature remained normal, appetite improved, insomnia lessened, and she regained 5 lb (2.3 kg). The patient declined a request to resume the former high intake of coffee to see if fever again appeared.

Effects of Caffeine

An ordinary cup of coffee contains about 0.1 gm of caffeine.[6,8] Accordingly, the amount ingested by the patient in 15 to 18 cups was between 1.5 gm and 1.8 gm or about 36 mg/kg. A single 1-gm dose of caffeine causes mental confusion, shivering, tremor, tachycardia, vomiting, and diarrhea, but the patient's intake was extended over a period of eight hours. A large therapeutic dose is 0.25 gm, and 10 gm is said to be fatal.[9] Caffeine is diuretic, rapidly metabolized and excreted. Excretion of large amounts may be delayed, but it is not cumulative.

In experimental studies, one hour after 0.5 gm of citrated caffeine was given, the temperatures of three persons rose 0.4 F, presumably as a result of stimulation of the hypothalamus.[5] Amounts of 0.25 gm to 0.4 gm given before retiring increased temperature and mobility at night.[10] Aminophylline (theophylline ethylenediamine) poisoning in children caused hyperpyrexia and albuminuria.[11] The basal metabolic rate increased from 7% to 23% after the ingestion of 0.65 gm of caffeine.[12]

Caffeine, a xanthine alkaloid, mainly stimulates the cerebral cortex, the thalamus, the vasomotor and respiratory centers, and evidently the thermal regulatory mechanism. It reduces the cerebral blood flow. Among the acute and chronic toxic effects are insomnia, irritability, cardiac palpitation, tremor, convulsions, flushing, anorexia, dehydration from diuresis, fever, albuminuria, and epigastric discomfort.[8] Seven of these features concerned my patient. The reflexes were not exaggerated, she had no tremor nor polyuria, but her habitual intake of phenobarbital and nicotine may have modified the action of caffeine. Moreover, her mildly psychoneurotic state may have played a role in her physical reactions. Variations in response also depend on whether a single large dose or repeated small doses are taken over a long period of time. Anyway, the nature and degree of reactions to caffeine are influenced by the age, the emotional or nervous state, or by the idiosyncrasies of people. Its action on physiologic functions is inconstant and variable, often diametrically opposite in different persons and even in the same person at different times.[12]

Caffeinism is said to be current among intellectual workers, actresses, waitresses, nocturnal employees,[13] and long-distance automobile drivers. Illness otherwise unexplained may be caused by excessive ingestion of the xanthine alkaloids, including those in coffee, tea, cocoa, and those in some popular beverages.

Generic and Trade Names of Drug

Phenobarbital—*Luminal*.

References

1. Reimann, H.A.; Ducanes, T.; and Supple, L.K.: Tricalcium Phosphate Crystallosis, *JAMA* 189:195-198 (July 20) 1964.
2. Lavers, G.D.: Peanuts for Luncheon, *JAMA* 201:332 (July 31) 1967.
3. Reimann, H.A.: Habitual Hyperthermia: A Clinical Study of Four Cases With Long Continued Low Grade Fever, *Arch Intern Med* 55:792-808 (May) 1935.
4. Reimann, H.A.: The Problem of Long Continued Low Grade Fever, *JAMA* 107:1089-1093 (Oct 3) 1936.
5. Friedman, M.: Etiology and Pathogenesis of Neurocirculatory Asthenia: I. Hyperthermia as One of the Manifestations of Neurocirculatory Asthenia, *War Med* 6:221-227 (Oct) 1944.
6. Landis, C.: *Physiological and Psychological Effects of the Use of Caffeine in Problems of Addiction and Habituation*, P.H. Hock, and J. Zubin, (eds.), New York: Grune & Stratton, Inc., 1958, pp 37-48.
7. Dreisbach, R.H., and Pfeiffer, C.: Caffeine Withdrawal Headache, *J Lab Clin Med* 28:1212-1219 (July) 1943.
8. Truitt, E.B.: "The Xanthines," in DiPalma, J.R. (ed.): *Drill's Pharmacology and Medicine*, New York: McGraw-Hill Book Co., Inc., 1965, pp 394-409.
9. Khodasevich, A.P.: Fatal Caffeine Poisoning, *Farmakol Toksik* 19 (suppl):62, 1956.
10. Mullin, F.J.; Kleitman, N.; and Cooperman, N.R.: Studies on the Physiology of Sleep: X. The Effect of Alcohol and Caffeine on Mobility and Body Temperature During Sleep, *Amer J Physiol* 106:478-487 (July 31) 1933.
11. Soifer, H.: Aminophylline Poisoning, *J Pediat* 50:657-660 (June) 1957.
12. Means, J.H.; Aub, J.C.; and Du Bois, E.F.: The Effect of Caffeine on the Heat Production, *Arch Intern Med* 19:832-839 (May) 1917.
13. Hesse, E.: *Narcotics and Drug Addiction*, New York: Philosophical Library, Inc., 1946, p 172.

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CARDIOVASCULAR ACTIONS OF CAFFEINE AND COFFEE IN ANIMALS AND MAN

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sur la chimie des cafés, Trieste, 1967

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During the past year we have had the opportunity to review the available literature on the biological effects of caffeine and coffee and to assess their relationship to health and disease. This report presents a brief summary of what we found in terms of cardiovascular effects and what we think it means. As is indicated, there are many gaps in our knowledge. The results of this review are reported in two general sections, the first dealing with data obtained in **animal experimentation** and the second with results derived from **observations in man.** The animal studies involving cardiovascular effects deal almost exclusively with caffeine. The data on man include caffeine and coffee and suggest biological activity for some as yet unidentified coffee components.

As a preface to the following sections, it is desirable to give a **brief summary of caffeine's cardiovascular effects,** as presented in a standard American textbook of Pharmacology.

Referring to the xanthine compounds generally, GOODMAN and GILMAN (1) note their ability to cause smooth muscle relaxation, coronary artery dilation and cardiac stimulation, with theophylline more potent than theobromine, and the latter more potent than caffeine. Systemic blood pressure is modified negligibly, or not at all, by the xanthines, because of opposing actions within the cardiovascular system. Thus, dilation of major vessels by xanthines would produce a fall in blood pressure were it not offset by myocardial stimulation. Simultaneous antagonistic effects within the central nervous system may also participate in this equilibrium.

Most major vascular beds are dilated by the xanthines according to GOODMAN and GILMAN (1), with the notable exception that the cerebral vessels of man are constricted. This last effect is believed responsible for reported relief of hypertensive headaches by xanthines.

C. J. KENSLER

Though xanthines can dilate coronary vessels, the demand imposed by concurrent myocardial stimulation may outstrip the increased blood supply. Controversy over the therapeutic value of xanthines in angina continues, but the consensus tends toward interdiction. Finally, and most important, it is established that tolerance, as well as cross-tolerance develops with continued use of the xanthines, particularly to the diuretic and vasodilator actions.

Specific references to caffeine are as follows : caffeine causes direct dilation of coronary, pulmonary, and general systemic vessels, but also stimulates the medullary vasomotor center tending thus to produce vasoconstriction. With therapeutic doses (0.25-0.5 gm) the dilator action predominates, and blood flow is increased because

of the concomitant rise in cardiac output. However, the flow increase is probably too little and too brief « to be of value in the treatment of peripheral vascular disease ». Caffeine toxicity may occur with amounts of 1 gm or more, and in this connection tachycardia and extrasystoles are frequent. Arrhythmias are also occasionally encountered « in persons who use caffeine beverages to excess », though the authors do not specify the meaning of « occasionally » and « excess ».

In subsequent sections only the most important references are cited in the text. However, a more complete reference list is included.

ANIMAL STUDIES

Effects on systemic blood pressure and heart rate in intact animals

SOLLMAN and PILCHER's (21-24) conclusions about the general cardiovascular effects of caffeine, reported in 1912, are essentially valid today. They administered caffeine intravenously (I. V.) (2-800 mg/kg) to anesthetized dogs and cats, and observed : « cardiac stimulation or depression according to the dose and rapidity of injection ; increased heart rate, not due to vagus depression (if the vagus is intact there may be slowing through central stimulation, but with large doses the heart escapes from vagus tone) ; vasodilation, through peripheral depression of the vasoconstrictor mechanism ; central vasoconstrictor stimulation, generally ineffectual ; and cardiac irregularities with large doses ».

Doses of caffeine to 20 mg/kg produced relatively slight cardiovascular effects, principally a lowering of blood pressure. The effects also depended on rate of injection and level of initial blood pressure (the fall being greater the higher the initial pressure). Increased heart rate, when it occurred, usually had a shorter duration than the vasodilator effect.

Separate phases of their intensive study demonstrated that the acute fall in blood pressure after intravenous caffeine was a direct result of peripheral vasodilation. That caffeine is not an inordinately potent vasodilator may be gauged by effects on the animal under intense vasoconstrictor drive. SOLLMAN and PILCHER found that caffeine doses of 75-200 mg/kg, I. V., were required to halve the pressor response to epinephrine or to reduce that due to carotid occlusion.

Subsequent animal studies have confirmed these conclusions. J. D. PILCHER (16) obtained an acute fall of blood pressure in dogs after rapid I. V. injection of caffeine, in doses to 20 mg/kg. After the fall, blood pressure usually increased by a few millimeters of mercury (mm Hg), concurrent with a slight increase in heart rate. At dose levels around 20 mg/kg, the average blood pressure increase was 17 mm Hg, and the average heart rate increase was 30 per minute. Dose dependence of the direction of blood pressure change is also indicated in WALTON and BRODIE's study (32) in anesthetized dogs. Intravenous caffeine citrate, 10 mg/kg, decreased blood pressure by 35 to 60 mm Hg, whereas 20 mg/kg produced an average increase of 6 mm Hg.

The phasic nature of blood pressure responses to intravenous caffeine, i.e., a fall followed by a rise, has also been reported in other studies on anesthetized animals (TSUBOI (29), rabbits, 0.5-5 mg/kg caffeine sodium benzoate ; HAHN (8), cats 3 mg/kg caffeine). In addition, SCHMID, *et al.* (19), have demonstrated this sequence in unanesthetized dogs with caffeine doses of 1-15 mg/kg.

Nevertheless, the secondary pressor response to intravenous caffeine is not always evident or prominent. Essentially pure decrease in systemic pressure occurred after I. V. caffeine in SALANT's study (18) (anesthetized dogs, 15-25 mg/kg) and those of JUNKMANN and STROSS (10-11) (urethanized rabbits, 5-10 mg/kg), TIFFENEAU and BEAUNE (28) (chloralosed dogs, 10-30 mg/kg), MARTINETTI (12-13) (chloralosed dogs, 1-20 mg/kg of caffeine sodium benzoate), and CRAVER, *et al.* (5) (anesthetized dogs, 1-50 mg/kg).

Use of denervation procedures and blocking drugs in these and other studies point unmistakably to a direct peripheral vasodilation by caffeine as the major cause of the blood pressure fall. The secondary blood pressure rise is more complex. The initial blood pressure drop would evoke, via the pressoreceptors, reflex vasoconstriction and cardiac stimulation. No doubt this response contributes to the transience of the initial vasodepression. In addition, direct cardiac effects of caffeine may be involved in the secondary pressor response, though SOLLMAN and PILCHER (21-24) noted that caffeine vasodilation outlasts cardiac stimulation. More likely, the secondary pressor response represents a central effect of caffeine as SOLLMAN and PILCHER (21-24) suspected. Thus, DIKSHIT (6), MARTINETTI (12-13) and HAHN, *et al.* (9) injected caffeine into the cerebral circulation, or intraventricularly, and obtained prominent pressor responses (as well as cardiac arrhythmias ; DIKSHIT) in dogs and cats. Relatively high doses were required (10-12 mg/kg, intraventricularly), which probably accounts for the dose-related appearance of the

pressor response after I. V. caffeine. The high « central » dose of caffeine needed for pressor responses also explains SOLLMAN and PILCHER's conclusion (21-24) that central vasomotor stimulation with I. V. caffeine is generally « ineffectual ». It may also be that the centrally-induced hypertension is only distinct when the pre-existing blood pressure level is low (JUNKMANN and STROSS 10-11).

In summary, the preeminent effect of I. V. caffeine in anesthetized animals is to decrease blood pressure and increase heart rate. Dose and route of administration are of overriding importance in the meaning of this generalization. Doses to 10 mg/kg, I. V., produce a slight to moderate fall in blood pressure, and slight increase in heart rate. But this route of administration presents the peak drug concentration instantaneously to the circulatory system. With slower drug absorption (as after oral administration) it can be predicted that, dose for dose, cardiovascular effects of caffeine would be even less, and that compensatory circulatory reflexes could keep pace, at least partially offsetting the caffeine-induced changes. Finally, it must be remembered here (and in nearly all animal studies) that the experimental population is one with no previous exposure to caffeine, and therefore represents an intolerant population.

Effects on cardiac function and structure

Alterations in cardiac rate and cardiac work (or force of contraction) have been the major focus of these studies. Somewhat less attention has been accorded to caffeine effects on cardiac rhythm.

FUNCTION IN ISOLATED HEARTS

Amphibian hearts have been a popular test object for caffeine studies (see References). But the results obtained have limited applicability to man, from the standpoint of dose. Reviews of caffeine investigations on frog heart (NEUROTH, *et al.* 60 ; LÜLLMAN 56) indicates that concentrations ranging between 1 : 40,000 and 1 : 100 have been used ; i.e., from 0.1 to 50 millimolar (mM) approximately. However, increase in the force of contraction (positive inotropic) and in heart rate (positive chronotropic) are only distinct at caffeine concentrations in the range of 0.5-1 mM. Assuming a distribution of caffeine in body water of man (35 liters), attainment of these concentrations would require a single caffeine dose, rapidly absorbed, of approximately 3.5 to 7 gm.

Similar considerations apply to studies with isolated mammalian hearts or heart-lung preparations. The dose threshold in isolated cat hearts, for positive chronotropic and inotropic effects, is a caffeine concentration of 0.5 mM (STAUB and GRASSMAN 70). Rabbits are even less sensitive, positive chronotropism being the only effect seen with 5 mM caffeine (STAUB and GRASSMAN 70). Cat papillary muscle appears somewhat more sensitive than isolated cat heart ; threshold positive inotropic effect can be obtained with caffeine, 0.12 mM (KUSCHINSKY and MUSCHOLL 53-54). The latter authors also found that theophylline is approximately four to five times more potent than caffeine. Furthermore, theophylline increased excitability (measured by strength-duration curves) of the cat auricle whereas caffeine was without effect on this parameter.

Heart-lung preparations (HLP) of cat, rat and dog have also been used to assay caffeine's effect on cardiac performance. LÜLLMAN's (56) investigation of caffeine in the rat HLP demonstrated positive chronotropic and inotropic effects in approximately half of many experiments with small to moderate doses (0.05-0.2 mg injected into the inflow close to the heart). No change, or a decrease in amplitude of contraction (negative inotropic) was observed in the rest. The magnitude of changes was small, and higher doses yielded mostly negative inotropic effects together with diminution or disappearance of the positive chronotropism. Most significant, the effects over much of the dose range did not lead to an increase in cardiac output ; but very high doses (1-2 mg) halved the output. One of the theophylline group of experiments, the only one to show an increase in cardiac output, provides an important clue ; HLP's in this group were made insufficient by barbiturate administration. Other authors have speculated that positive inotropic and chronotropic effects of the xanthines, caffeine especially, are only prominent in insufficient, failing, or spontaneously weakened hearts (BRUNS and ROSENCRANTZ 39), and this suspicion will again be evident below.

FUNCTION IN HEARTS OF INTACT ANIMALS

J. D. PILCHER (89) explored a wide range of caffeine dosage in anesthetized, open-chest dogs, utilizing a ventricular plethysmograph and myocardiograph. His experiments are of value principally in support of LÜLLMAN's (56) observation that positive chronotropic effects of caffeine are obtained more regularly than are positive inotropic. Caffeine, I. V., to 20 mg/kg, usually increased blood pressure and heart rate moderately, but the amplitude of cardiac excursions was unaffected or only slightly increased (PILCHER 89). Effects of I. V. caffeine, 4-10 mg/kg, on the rate parameter also predominated in SALANT and NADLER's study (93) in dogs, cats and rabbits.

JOURDAN, *et al.* (87) and SCHAFF and FAUCON (94) established that xanthine effects on heart are largely direct ; also that caffeine « possesses only feebly the properties of aminophylline ». Chloralosed dogs with acutely denervated hearts and adrenal vessels ligated were given caffeine, 8-16 mg/kg, or aminophylline, 8-12 mg/kg I. V. Caffeine still increased contractile force and heart rate by 15 and 7 % respectively, with an average effect duration of approximately 15 minutes. Aminophylline figures, in the same sequence, were 46 and 33 %, and 17 minutes.

Anesthesia is, of course, a potentially complicating factor in animal studies. C. PILCHER, *et al.* (89) attempted to avoid this by measuring cardiac output (FICK principle) in trained unanesthetized dogs. Some of the animals received morphine prior to the experiment. I. V. and subcutaneous caffeine sodium benzoate, 7.5, 15 and 30 mg/kg were employed, which correspond approximately to total doses of caffeine base, in man, of 0.25, 0.5 and 1.0 gm. Six minutes after the I. V. and 30 minutes after the subcutaneous administration of the two lower doses, cardiac output was reduced less than 10 % on the average, within the limits of error. Caffeine, 30 mg/kg, caused a larger decrease in cardiac output.

Contrary results were obtained by SCHMID, *et al.* (19) in two unanesthetized dogs. Caffeine, 7 and 12 mg/kg, I. V., caused a slight increase in cardiac minute volume (output). These doses are equivalent to 0.5 and 0.8 gm, total, in man. The effect on cardiac output occurred in conjunction with a fall in blood pressure, tachycardia and reduction in peripheral resistance. Succeeding this was a phase in which blood pressure and peripheral resistance increased, probably reflexly.

In summary, experiments in intact animals support the observations on isolated cardiac tissue that moderate doses of caffeine elicit positive chronotropic effects of slight magnitude. Whether inotropic effects occur, whether they are positive or negative, and of what degree, depend largely on dose, route of administration, and state of the heart and circulatory system. One may note again that caffeine doses, given I. V., producing little or no change in cardiac output of animals, represent peak instantaneous blood and tissue levels beyond those that man might reasonably be expected to encounter in other than therapeutic situations.

CARDIAC ARRHYTHMIAS

Irregularities in cardiac rhythm can occur via many different mechanisms. Principal ones include changes (in either auricle, ventricle or specialized conduction tissue) in refractory period, conduction time, conduction pathway, excitability, or automaticity. Drug-induced arrhythmias in the intact animal reflect the direct and/or indirect production of one or more such changes. Caffeine is no exception. Direct effects of caffeine on cardiac rhythm have been demonstrated in chick embryo (BRINLEY 38) where 10 mM caffeine causes A-V dissociation. 1.0 and 0.1 mM solutions produce irregularities of the ventricle after long soaking. CHENEY (101), however, found that caffeine concentrations of 25 mM and higher were required to produce rhythm irregularities in the toad ventricle strip. Mechanisms were not examined in this study, but GIES (105) has shown that caffeine, 1-5 mM, lengthens the refractory period of frog heart. Caffeine also may increase the conduction velocity in frog heart (BENTHE 99), though this requires increased potassium concentrations and 4 mM caffeine.

Other work in animals sheds only minimal light on the mechanism of caffeine arrhythmias. KUSCHINSKY and MUSCHOLL (54) measured a 5 % decrease in the refractory period of cat papillary muscle, exposed to 0.12 mM caffeine. One cannot say whether this is an absolute difference from effects on the frog heart (GIES 105) noted above, or a difference due to dose. Arguments in favor of dose dependence could be adduced from experiments on guinea pig atria (de GUBAREFF and SLEATOR 103). Action potential duration was distinctly increased only at caffeine concentrations of 2.5 and 5 mM, and this situation would necessarily increase the refractory period. If conduction velocity were also increased, as in frogs (BENTHE 99), the conducted impulse in the atria might take aberrant pathways, or possibly ectopic foci might prevail. The ventricle obviously represents a different situation, for here action potential duration was not increased by caffeine (de GUBAREFF and SLEATOR 103).

As the preceding data indicate, high concentrations of caffeine are generally necessary to alter electrical parameters of cardiac function. Such is also the case in intact animals. SOLLMAN and PILCHER (21-24) observed that arrhythmias in the intact unanesthetized dog may be sudden in onset but usually require doses greater than 20 mg/kg, I. V., for provocation. BEATTIE, *et al.* (98) produced extrasystoles in cats with I. V. caffeine at levels of 35-45 mg/kg, and UEDA, *et al.* (112) initiated cardiac irregularities in anesthetized dogs with I. V. caffeine in the dose range 25-100 mg/kg. This study was notable in showing that the arrhythmias did not occur after caffeine in spinal dogs. Considering DIKSHIT's report (6) that intraventricular (cerebral) injection of caffeine, 10-12 mg/kg, produced pressor responses and arrhythmias in chloralosed cats, we must conclude that caffeine may also induce arrhythmias indirectly via actions on cardiovascular control centers in the central nervous system.

Nevertheless, both direct and indirect modes of arrhythmia production by caffeine entail the use of extremely large doses. With regard to man, extrapolation of the above doses in intact animals stands as the equivalent of approximately 10-15 cups of coffee at one time.

Interaction of caffeine with other drugs capable of either producing or controlling arrhythmias has also been studied. Most pertinent are epinephrine and cardiac glycosides. Caffeine may diminish epinephrine-induced arrhythmias or prevent the fatal ventricular fibrillation of epinephrine-hydrocarbon anesthetics (DAUTREBANDE and CHARLIER 102 ; MARTINETTI 12-13). The mechanism apparently centers on caffeine hypotension preventing the pressor response to epinephrine. Time and dose factors are critical, for others have observed augmented arrhythmias with caffeine-epinephrine combinations (UEDA, *et al.* 112 ; MEZZASALMA 108).

Caffeine-digitalis interactions have yielded conflicting results. PREOBRAZHENSKII (110) found I. V. caffeine, 2-50 mg/kg, to raise the minimal lethal dose (MLD) of strophanthin in frogs and cats, depending on the manner of administration of caffeine. HAAG and WOODLEY (106)

showed, in cats, reduction of MLD's of cardiac glycosides, but noted that results are a function of caffeine dose and the particular digitalis body.

This field is therefore controversial, but perhaps its importance is minimized by HAAG and WOODLEY's observation that caffeine doses approaching 50 % of the acute fatal dose are necessary to decrease MLD's of digitalis.

PATHOLOGICAL CHANGES IN THE HEART AND MAJOR VESSELS

Microphysical changes in myofibrils of frog heart followed a 30-minute soak in 2 % caffeine (CHENEY 114). The nature of the change was a clot formation within some of the myofibrils. Since a 2.2 % solution of caffeine is saturated, the above results have no practical significance. However, the author notes that cardiac muscle is much more resistant than skeletal muscle in which a 0.03 % solution of caffeine produces clot formation in ten minutes.

LEVI and CABRINI (118) claimed that caffeine citrate administration (10 or 40 mg/kg/day) for 30 days to rabbits led to vascular alterations in the capillaries, and slight fatty degeneration in a few myocardial fibers, especially at the higher dose level. Unfortunately, their report does not indicate whether histological examinations were also done on the controls. However, WENZEL, *et al.* (124) failed to detect myocarditis in rabbits given caffeine, 5 mg/kg I. V., once a week for 24 weeks. Nor did this regimen enhance subintimal lipid deposition in the small-medium coronary arteries when the animals were on a hypercholesterolemic diet. Indeed, SERANOVA (122) showed that 80 days of caffeine administration (either 0.6 mg/kg or 100 mg total, subcutaneously) together with oral cholesterol yielded cholesterol blood levels lower than those in animals receiving only cholesterol. Blood cholesterol levels within the caffeine group were, however, higher in the animals receiving the larger caffeine dose. Surprisingly, aortic atherosclerotic changes were less, within the caffeine group, at the higher caffeine dose level. This work confirms earlier, identical conclusions (SHKHVATSABAYA 123 ; CZOCHRA-LYSANOWICZ, *et. al.* 115).

Effects on specific vascular beds

PERIPHERAL, VISCERAL AND PULMONARY VESSELS

Evidence, neither abundant nor detailed, exists to show that caffeine directly dilates these vessels ; it is further known that the intensity of effect differs from bed to bed, and it also depends on the preexisting tonus state of the vessels.

SOLLMAN and PILCHER's (21-24) plethysmographic studies revealed dilation of peripheral, intestinal, splenic and renal vessels by caffeine. The order of effect intensity in the viscera was intestinal > splenic ≥ renal, with the intestinal vasodilation outlasting the systemic blood pressure change. In these perfusions of isolated organs it was necessary, however, to heighten vasoconstriction by means of epinephrine in order to demonstrate dilator actions of caffeine. Under these conditions 0.5 mM caffeine gave a distinct renal vasodilation. High doses (20 mg, intraarterially) for dilation of renal and paw vessels of dog were also the case in BECQ and PLUMIER's study (127).

A number of Japanese investigators have demonstrated dilating effects of caffeine on isolated mesenteric arteries of frog, cat and cow ; for example, FUJIMORI (132) with 0.5 and 5 mM caffeine in bovine vessels. Veins were little affected.

Other studies (see References), indicating caffeine effects on lymph vessels and veins, are insufficiently detailed to warrant conclusions.

Similarly, the pulmonary vessels have received scant attention with respect to caffeine action. More information is available on xanthines other than caffeine (aminophylline, for example), from which it may be inferred that caffeine would differ only in potency. The recent study of QUIMBY, *et al.* (151) typifies this situation ; caffeine was part of an investigation of 40 xanthines in anesthetized dogs, and it is stated that caffeine was a potent pulmonary vasodilator. Two other significant findings emerged : pulmonary and leg vessels reacted in qualitatively similar fashion to a given xanthine ; and caffeine was more potent than aminophylline as a pulmonary vasodilator but weaker as a dilator of femoral vessels.

CEREBRAL VESSELS

Reviewing drug action on the cerebral circulation, SOKOLOFF (171) noted that conflicting evidence for vasoconstriction and vasodilation exists in animal studies with xanthines ; the result obtained depends on dose, animal, systemic pressure changes, anesthesia, etc. Examples of the conflict are reflected in key studies in this field. Finesenger (162) explored the caffeine dose range 2.5-63 mg/kg I. V. in cats anesthetized with amytal, wherein he obtained roughly dose-related increases in diameter of the pial artery. Coincidentally, systemic blood pressure fell and cerebrospinal fluid pressure rose. Vasodilation was not obtained when the experiments were repeated under ether anesthesia, instead slight vasoconstriction occured. After recovery from ether, caffeine elicited a brief constriction followed by a longlasting dilation. Here is a classic illustration of caffeine's ultimate effect depending on preexisting vascular tonus. Ether dilates cerebral vessels (caffeine thus constricts) and its elimination is followed by marked constriction (caffeine thus dilates).

SCHNEIDER (170) measured an increase, then a decrease, in carotid blood flow in anesthetized dogs after caffeine sodium benzoate, 2-4 mg/kg, I. V. But carotid blood flow reflects extracranial as well as intracranial changes, and is therefore not an ideal measure of cerebral circulation. DUMKE and SCHMIDT (161),

however, used this measure in their classic examination of cerebral blood flow in monkeys anesthetized with pentobarbital. Cerebral blood flow was increased only slightly in three of five animals by caffeine, I. V., approximately 2-5 mg/kg. Intraarterial caffeine, 6 mg, elicited a flow increase with rapid onset and a duration of three to four minutes. GOTTSTEIN, *et al.* (183) confirmed this by intraarterial injection of caffeine (0.5-1 mg/kg) in anesthetized dogs. However, intravenous administration (1-3 mg/kg) did not alter blood flow. Higher doses decreased flow in connection with the depressed blood pressure.

Thus, SOKOLOFF's views (17) are substantiated, and the numerous factors in the discordant results are evident. Beside those mentioned previously, one might also consider nonhomogeneous effects of caffeine in the cerebral vascular bed. Recent work of BIRZIS, *et al.* (158) demonstrated an increase in cortical, but a decrease in subcortical, blood flow after intraperitoneal caffeine, 25 mg/kg, in unanesthetized cats.

CORONARY VESSELS

The technical difficulties of analysing coronary flow changes in the beating heart are well known. Control of rate and amplitude of contraction, or elimination of activity as in the use of fibrillating hearts, represent possible solutions, albeit unsatisfactory. Another approach is the dissociation of mechanical and flow events by careful attention to dose considerations. Such was the method used by STAUB and GRASSMAN (70), who also summarized the earlier literature in this field. Coronary flow in isolated rabbit hearts was increased, throughout the caffeine concentration range of 0.05-5 mM and was separable, by dose, from the caffeine effects on rate and amplitude. Dissociation was not possible in cats, however, for all three parameters had the same caffeine threshold (0.5 mM). Except for concentration, this confirms IWAI and SASSA's earlier study (177) of isolated cat hearts.

Isolated hearts may be more favorable for displaying coronary vasodilation by caffeine than those of intact animals. And even in the isolated heart the pre-existing tonus of coronary vessels is a critical factor as shown superbly by HEIMANN and WILLBRANDT (175) for the guinea pig heart.

No less important is the distinction between experiments in dogs with open chests and closed chests. JOURDAN and FAUCON (178) reported a 20 % increase in coronary flow in chloralosed open-chest dogs after I. V. caffeine, 5-7 mg/kg. Though this occurred without an increase in heart rate, the results are marred by the fact that the dogs were in shock. Much more skill is evident in the work of LOCHNER, *et al.* (180) on anesthetized dogs with closed hearts. Oxygen saturation of coronary sinus blood was used as the indicator of coronary flow, and it was unaltered by caffeine doses to 5 mg/kg. LOCHNER, *et al.*, concluded that « higher doses did not appear of interest to us — 10 mg/kg is 0.7 gm in a 70 kg man ; with this dose there appear strong central nervous system effects ».

Thus the strong belief that caffeine dilates coronary vessels rests largely on experiments with isolated hearts. Some studies in intact animals support this belief, but are not unequivocal. The sum of present data suggests then that caffeine produces either no change or an increase in coronary blood flow ; and it remains to be seen whether any increase is always overcome by the increase in cardiac work.

STUDIES IN MAN

CAFFEINE AND COFFEE

Interest in the cardiovascular effects of these substances in man is relevant to two areas of concern. One is the purely therapeutic ; does caffeine have value in the treatment of one or more cardiovascular or cardiopulmonary disease states ? The other area has much broader implications ; is coffee-drinking beneficial, indifferent, or harmful with respect to the cardiovascular system ? And, should coffee-drinking be prescribed or proscribed in some normal individuals, or inthose with certain cardiovascular diseases ? Moreover, it is apparent that many investigators make the simple equation that cardiovascular effects of coffee are those of caffeine. This appears to be substantially true, for acute effects at least, but there is little knowledge in animals or man about actions of the other coffee components.

In the following sections, where the amount and quality of literature warrants, caffeine will be considered first, followed by results with coffee. Investigations with caffeine frequently utilize one of the several salts of this alkaloid ; sodium benzoate, salicylate or citrate. To simplify the presentation, however, all doses are expressed in terms of the base.

Effects on blood pressure and heart rate

CAFFEINE

Intravenous administration

In the period 1931-1960, thirteen investigations provided data about these parameters. The experimental population was composed of normal subjects and hospitalized patients with and without cardiovascular disease. A wide age span was represented. Dosage ranged from 0.1 to 0.5 gm, though the predominant part of the population was explored with doses of 0.1 to 0.25 gm. In all, reactions were observed in 132 normotensive individuals and more than 26 with hypertensive cardiovascular disease.

With rare exception the consensus of these studies is that caffeine, within the above dose range, produces relatively slight variations in blood pressure and heart rate. Nor is the direction of variation consistent. This could be attributed to normal population variations, with respect to both general cardiovascular reactivity and responsiveness to caffeine ; plus the fact that time course of caffeine injection varied widely.

Examples of low dose studies are those of DENKER (200) and FUCHS (201) in normotensive individuals. The former administered 0.15 gm caffeine to each of 50 subjects and observed no marked pulse or blood pressure changes. FUCHS (201), however, found that pulse slowing (10 beats, minute) and blood pressure decrease (18 mm Hg systolic, 14 mm Hg diastolic) occurred within 10 seconds after injection in four young men. Recovery to initial levels ensued within 1-2 min. Both investigations lacked adequate controls.

Intermediate doses were used by BRANDT (198) and GIBBS (202), among others. Twenty-eight normotensive patients showed no blood pressure change, and a very slight increase in heart rate after 0.2 gm caffeine (BRANDT 198). Measurements were made in the period five to twenty minutes after injection. BRANDT was one of the very few to record venous pressure changes. Seventy per cent of the subjects manifested a decrease, averaging 20 mm H_2O, the remainder had an average increase of 13 mm H_2O. GIBBS (202) injected 0.25 gm caffeine rapidly (30 seconds) in six normotensive patients who responded only with a slight increase of systemic blood pressure. One might compare this with the study of MOYER, *et al.* (213) in which a slow injection (ten minutes) of 0.25 gm caffeine produced slight pressure rise (systolic 11 mm Hg, diastolic 4 mm Hg, average) in seven patients, five having hypertensive cardiovascular disease. Venous pressure and heart rate were essentially unchanged. SHENKIN (219), using the same dose in nine normotensive patients, observed an average blood pressure increase of 8 mm Hg (systolic) ($P < 0.02$). MAKINO (208), however, found a blood pressure decrease averaging 4 mm Hg in eight patients and this was not statistically significant.

Six normotensive patients examined by LOMAN and MYERSON (206) received 0.5 gm caffeine. They illustrate the population variation factor, in that : one subject exhibited an initial blood pressure rise followed by a slight fall ; blood pressure was elevated in one ; two showed a transient decrease, and blood pressure was unchanged in the remaining two. Venous pressure again showed little or no shift.

One subject in one study requires special comment. BÖGER, *et al.* (197) administered caffeine, 0.15 gm, rapidly to a 34-year-old healthy male. Within one minute blood pressure had increased by 44 mm Hg and the heart rate accelerated by 45 beats per minute. Recovery proceeded over the next five minutes. The author noted that this is not a « typical » reaction to caffeine, but presented it to illustrate the similarity in cardiovascular reactions to epinephrine and caffeine. Since this was also the most extreme reaction among the 132 normotensive subjects in the thirteen studies, it is concluded that this individual belongs either to one tail of the normal population distribution or to a separate hypersensitive population.

Intramuscular and subcutaneous administration

Of the three investigations in which this route has been employed, that of STARR, *et al.* (220) is still the classic reference. Measurements of many parameters of cardiovascular function were made in four patients, 20 to 40 minutes after intramuscular caffeine, 0.5 gm. The average decreases in heart rate and blood pressure, 2.5 % and 4 % respectively, were not statistically significant. Indeed, STARR, *et al.* (220) point out that the absence of marked pulse rate and blood pressure effects gave them no guidepost for selecting the optimal time to measure other cardiovascular functions.

Oral administration

Beside the studies in which oral caffeine and coffee have been compared stands another group in which caffeine alone was investigated. One of these (WOOD 231) superb for its recognition of the many extraneous factors modifying the experimental situation, is also the first, and one of the few, to be aware that the subject's caffeine-drinking practices might be relevant to the outcome. Two of WOOD's subjects were abstainers and two consumed coffee regularly. Caffeine, 0.08-0.36 gm, or an identical capsule containing placebo, was given. Very slight pulse slowing and an average blood pressure increase of 2 mm Hg developed after the caffeine, peaking at 45 minutes and returning to baseline approximately two hours after drug. The maximum blood pressure increase, shown by one individual, was about 9 mm Hg. Differences between coffee users and nonusers were slight, and Wood noted further that each individual's type of response was consistent in repeat trials.

GROLLMAN's experiments (204) on four healthy subjects demonstrate the significance of dose. Caffeine,

0.16 and 0.32 gm, left the pulse rate and blood pressure comparatively unaffected, whereas 0.65 gm and 0.97 gm produced small but significant (0.97 gm) increases in both measures. Peak effects occurred 10-40 minutes after drug, obviously an absorption-determined characteristic, and declined gradually to control within 2 hours.

At the other extreme, low dosage, SEBOK's work (217) is the sole example. Caffeine was given to seven normotensive and thirty-two hypertensive patients, in doses of 0.05 and 0.1 gm. Blood pressure dropped 5-35 mm Hg in the normals, and the decrease was greater in hypertensives. Pulse rate changes were slight or absent in both groups. Lack of controls mars this study, but time course of drug effect is in line with those noted above. It is possible that SEBOK's small doses of caffeine have achieved dissociation of vascular and cardiac effects, and that dose-effect curves at these two sites may be slightly more separated in hypertensives than in normotensives.

COFFEE AND CAFFEINE

The minimal crossover design in experiments to evaluate cardiovascular effects of coffee would be comparison of the beverage with decaffeinated coffee and caffeine. Addition of placebo to this regimen constitutes the optimal design.

HORST and co-workers (229) met the minimal design requirements but the studies suffered from the great variation in the times at which effects were measured. Several very general conclusions appear warranted, however, referable to the dose investigated, 3-4 mg/kg (coffee and caffeine). Blood pressure and pulse changes in young healthy subjects were slight, but generally an increase seldom exceeding 10 mm Hg in the former and a decrease averaging 5 per minute in the latter. These effects, not duplicated by decaffeinated coffee (hereafter designated as decaff), tended to diminish on repeat testing in subsequent weeks of study. Individual differences were evident, such that decaff and coffee had identical effects in some subjects. Evaluation of the age factor suggested that older men (53-77 years) showed a greater blood pressure rise than young men (21-25 years) after coffee ; but half of the older subjects had very high control blood pressure levels.

These studies also illustrate a failing typical of many investigations in this area ; lack of information about coffee habits of the subjects. This deficiency would minimize the work of VALLE and MELLO (234) and FLEISCH and WENNER (228) were there not compensation in the materials studied. VALLE and MELLO (234) examined coffee, decaff and decaff plus trigonelline in 18 normal subjects ; the caffeine and trigonelline doses were approximately 2-4 mg/kg. All three beverages produced very small peak effects, averaging only 1-3 % for pulse rate decreases and blood pressure increases.

Equally small, but statistically significant, changes were found in FLEISCH and WENNER's study (228) of 25 normal subjects. Comparing filtered and soluble coffee (caffeine, 0.34 and 0.24 respectively) they observed that most subjects had maximum reactions one hour after ingestion. Pulse rate declined 6-7 beats per minute with all beverages, but blood pressure increased 3-4 mm Hg with the two coffee preparations. Decaff lowered blood pressure about 2 mm Hg (not significant).

BUHR's report (224) represents the only instance of optimal experimental design, and is enhanced by the division of subjects into coffee users and non-users. Twenty-one of the sixty subjects were hypertensive, the remainder normotensive and those with several forms of heart disease, but not decompensated. Coffee, caffeine and brown colored water were given as a volume of 300 ml, the caffeine content (coffee, caffeine) being 0.22 gm. Normotensive patients accustomed to coffee showed no significant change in heart rate after coffee or caffeine. Those not accustomed to coffee exhibited an increase in both blood pressure and heart rate, maximum 15 minutes after coffee and caffeine. Decaff and control solutions produced no effect or a slight reduction of the parameters. Pulse rate and blood pressure changes in the hypertensive and cardiac patients are not described in detail. BUHR's discussion (224) cites several papers with conflicting results on the blood pressure effects of caffeine/coffee in hypertensive patients. Clearly, this area merits further and more careful study.

Effects on cardiac function

CARDIAC OUTPUT — CAFFEINE AND COFFEE

Cardiac output, i. e., the volume of blood delivered per stroke or per unit time, may be measured in man in a variety of ways, none of which is absolutely ideal. Dye dilution, gas uptake and the sphygmographic method of WEZLER and BOGER (197) are the more common, the last named perhaps having slightly more error than the other two.

Three representative studies of cardiac output, and the effects of caffeine alone, are those of MEANS and NEWBURGH (212), GROLLMAN (204), and STARR, *et al.* (220). GROLLMAN (204) criticized the MEANS and NEWBURGH experiments, justifiably, for the execution of control and drug runs on different days. In GROLLMAN's experiments (acetylene method) oral caffeine increased cardiac output in young healthy subjects by 15 % after 0.65 gm, and by 11 % after 0.97 gm. With 0.16 and 0.32 gm there were no measurable changes in output. The time course of drug action showed a peak at 10 minutes and a gradual recovery over 1-2 hours, similar to the time course of blood pressure effects noted earlier.

GROLLMAN (204) used one subject per dose, whereas STARR, *et al.* (220) used the same dose (0.5 gm caffeine, intramuscularly) in each of four patients, including one with hypertensive heart disease. The average

increase in cardiac output (15 %) was not statistically significant. Estimation of left ventricular work and heart volume revealed no significant change after caffeine.

Reference was made previously to a highly sensitive subject with dramatic blood pressure increase after caffeine (BÖGER, *et al.* 197). One to two minutes after intravenous caffeine, 0.15 gm, this subject's cardiac output rose by 50-60 %. Partial recovery had occurred after six to seven minutes. Cardiac work was calculated to have doubled.

Only two worthwhile studies have compared the effects of coffee and caffeine on cardiac output. BUHR's report (224), details of which are described in the preceding section, shows that decaff and a water control beverage do not increase cardiac output in normotensive patients and those with hypertensive and other forms of heart disease, regardless of their coffee habits. Cardiac output and cardiac work in normotensives accustomed to coffee were unaffected by caffeine or coffee (0.22 gm caffeine). Non-accustomed normotensives, however, manifested a peak 40-50 % increase in cardiac output 15 minutes after either caffeine or coffee. Similar effects were obtained in hypertensives, both accustomed and non-accustomed, though the degree of change was smaller in coffee-users. Patients with heart disease, and accustomed to coffee, were intermediate (20-30 % increase) in their reaction with respect to normotensives and hypertensives. The report does not make clear what level of effect is significant.

The lack of effect of BUHR's (224) control solutions, water and decaff, suggests that his sample population was an average one. This important function of control data, missing in so many caffeine and coffee studies, is emphasized by the second cardiac output report (NEUTHARD and HOEN 239). NEUTHARD was the subject, described as « sensitive to coffee », and cardiac output was determined by the acetylene method. Ten minutes after 0.1 gm caffeine orally, cardiac output had tripled ! Decline from this high level was slow, and three hours after drug the output was 20 % greater than control. During the peak response, and for two hours, subjective sensations were especially marked : pressure and fullness in the head, pulsation in particular vessels, tremors of the hands and twitching of small muscles (orbicularis oculi, fingers).

Theobromine and theophylline, orally, induced similar but less marked elevations of cardiac output, differing from caffeine in time course.

Coffee with caffeine content of 0.1 gm duplicated the effect of caffeine. Even decaff and malt coffee led to a doubling of cardiac output, as did ingestion of 200 ml of hot water, but the latter effect dissipated rapidly.

The NEUTHARD and HOEN paper (239) therefore reveals another complexity in interpreting cardiovascular reactions to drinking coffee, namely, cardiovascular responses to temperature or volume of the ingested beverage. Explorations in this area were summarized briefly by DRETTNER (236) who concludes that volume effects (vasoconstriction and vasodilation) are more prominent than temperature effects.

CARDIAC ARRHYTMIAS

Experimental production of arrhythmias in man, by caffeine, is obviously not a feasible or desirable approach. Consequently, knowledge in this area is somewhat limited and has been gained indirectly. The cases reported by REED, *et al.* (247) and BENNETT and WALKER (242), for caffeine, are irrelevant since these arrhythmias occurred in patients being treated for barbiturate and narcotic poisoning ; and other central nervous system stimulants were also being used in the treatment regimen.

One should note, however, that nearly all cardiovascular studies of caffeine and coffee in man have included EKG recording. Only very rarely have EKG alterations been mentioned.

MATHIEU, *et al.* (244) have reported their extensive experience with cases of rhythm disturbances in relation to coffee-drinking, drawn from a large population of night workers, principally railroad employees. The authors point out the importance of individual variation in the population, and the importance of distinguishing between tolerant and intolerant populations. Their major findings concern prolonged abuse of coffee, or intoxication, in 113 individuals. In this series the minimum daily intake of coffee was 4-6 cups. But size of the cup varied widely and MATHIEU, *et al.* found the modal volume to be 1 liter, with the distribution tailing to as much as 3 liters per day.

Tachycardia, though not literally an arrhythmia, was present in 24 of the 113 (24/113) subjects, with rates of 105-120/minute common at rest. Bradycardia (44-56/minutes) only appeared in 7/113.

Extrasystoles, evident in 26/113, were predominantly ventricular, and usually unaccompanied by other rhythm disturbances. Less frequently seen were complete arrhythmias (8/113) and auricular ectopic beats (8/113). Though associated factors (smoking, drugs) were recognized as possibly contributory, the relation to coffee was established by noting that nearly all arrhythmias resolved rapidly after reducing or stopping coffee intake.

The work of POLONOVSKI, *et al.* (246) offers the first real clue to the possibility that all cardiovascular reactions to coffee may not be due to its caffeine content. Their comparisons of coffee, decaff, and soluble extracts also included a division into lightly and highly roasted material. Eleven of 261 patients exhibited extrasystoles one-half hour after drinking the highly roasted material, including one out of 36 patients given highly roasted decaff. Extrasystoles did not occur in the 41 patients given lightly roasted coffee or lightly roasted soluble extracts.

The role of caffeine was tested further. A patient « sensitive to coffee » developed extrasystoles while

on coffee (caffeine, 0.2 gm), but not when given 0.2 gm caffeine in soup. Another patient not sensitive to coffee was given soluble coffee for ten-day periods in the following sequence : highly, lightly, and then highly roasted. Extrasystoles appeared only during the first and last periods.

Since DALLA TORRE and BOLDRINI (227) were also able to produce extrasystoles with decaff, it is conceivable that something other than the caffeine in coffee is responsible, either by itself or as a potentiator of caffeine.

Effects on specific vascular beds

Surprisingly little quality information exists about the actions of coffee or caffeine on specific vascular beds, with the exception of the cerebral circulation.

A decrease in cerebral blood flow after 0.25 gm caffeine, I. V., was observed by GIBBS, *et al.* (202) in five of six patients with epilepsy. The thermoelectric flow recorder allowed only qualitative indication of cerebral vasoconstriction. Stimulation of respiration also occurred, and the authors questioned whether cerebral vasoconstriction by caffeine was secondary to decreased pCO_2. DENKER (200) and LOMAN and MYERSON (206) inferred cerebral vasoconstriction from recordings of cerebrospinal fluid pressure, which declined after caffeine, 0.15, 0.25 and 0.5 gm, I. V. Fifty patients were tested with the 0.15 gm dose in DENKER's study (200) and the average peak reduction in CSF pressure was 36 % (range 8-68 %), occurring 8 minutes after drug and returning to control levels 30 minutes later. Since systemic blood pressure did not change materially the results point to cerebral vasoconstriction.

Three more recent studies, with the Kety-Schmidt nitrous oxide method, agree that caffeine can cause cerebral vasoconstriction and thus decrease cerebral blood flow. SHENKIN (219) obtained an average peak decrease in cerebral blood flow of 7 ml/100 gm/minute ($P < 0.02$) in nine patients given 0.23 gm caffeine, I. V. The same dose and route produced a significant ($P < 0.05$) decrease (25 %) in cerebral blood flow in MAKINO's study (208) of eight patients. Systemic blood pressure increased (8 mm Hg) significantly in SHENKIN's trials, but not in MAKINO's. Patients with hypertension also experienced a decrease in cerebral blood flow (concurrently with reduction in CSF pressure) after slow intravenous caffeine, 0.25 gm (MOYER, *et al.* 213). The average flow decrease, 7 ml/100 gm/min., was the same as SHENKIN (219) observed but was not significant ($P = 0.12$) in this series of seven patients. Most important, morning hypertensive headaches were completely relieved by the caffeine injection in four of the patients, and diminished in the remaining three. Relief by caffeine of the morning headache associated with hypertension is documented elsewhere as well (FUORTES and MASPES 253 ; DMITRENKO 250 ; SOKOLOFF 171 ; LIEBERMAN 256). A possible corrolary may exist in the caffeine withdrawal headache (DREISBACH and PFEIFFER 251).

It is not known what mechanism underlies the morning headache of hypertensive individuals, though venous stasis and/or postarteriolar dilation have been suggested. In any event, the question of whether caffeine or coffee is indicated for relief depends on how these agents affect the rest of the circulatory system. Thus it would seem desirable to settle the controversy about their effects on blood pressure and cardiac function in hypertensives.

Effects of caffeine on the human coronary circulation are virtually unknown. Studies in isolated human hearts (KOUNTZ 255 ; TRUSSEWITCH 264) have dubious, if any, value. One is forced to rely on extrapolation from animal studies, or on results obtained with aminophylline. The latter may either dilate or constrict coronary vessels in man, concurrently with positive inotropic effects on the heart ; hence the increase in cardiac work may outstrip any increase in coronary flow. MODELL (258) has summarized the field, concluding that aminophylline and other xanthines have « failed to survive trial under clinical fire ».

Coffee, caffeine and coronary artery disease

Coronary artery disease (CAD) is a general diagnostic classification encompassing entities such as angina pectoris and myocardial infarction. The etiology of CAD is obscure and therefore controversial. Most authorities agree, however, that etiology is multifactorial. Briefly, the pathological lesion is a deposition of lipid material (Atheromata) in the intimal portion of the coronary vessels leading to narrowing, rigidity, and/or occlusion. Symptoms develop as the oxygen supply of the myocardium is compromised, hence the more recent substitution of the term « ischemic heart disease » for CAD. Whether preceding vascular injury is a necessary condition for the development of atheromatous plaques is also a controversial question.

In an attempt to pinpoint etiologic factors, PAUL *et al.* (277) conducted a long-term study of a relatively stable population, numbering almost 2,000 men between the ages of 40 and 55. The men, moderately uniform ethnically, were studied carefully for nearly five years ; relevant data included family history, body type, regular physical examination, laboratory tests, diet, and other environmental factors. At the end of the study period, ischemic heart disease had developed in 88 men, of whom 13 had died as a result. Comparison of the data from the 88 (coronary group) with that from the remainder of the population (noncoronary) revealed several statistically significant correlations. In the coronary group systemic blood pressure, body fatness, blood cholesterol levels, cigarette consumption, and monthly coffee intake, were all greater than in the

noncoronary group. Additional significant correlations in the coronary group were histories of peptic ulcer, chronic cough, shortness of breath, chest discomfort and age of death of father.

The authors' discussion of their findings is responsible and objective, as evidenced, for example, by the use of the term « association » for the significant correlations. Association does not necessarily mean a cause-effect relationship. The authors are also obviously aware that in multifactorial associations such as this there are undoubtedly interactions between the separate factors. In other words, any single factor may not be significant unless one, two or more of the other factors are present. Therefore, it would be unjustifiable to quibble about any single correlation in this carefully performed study.

Subsequently, BELLET and co-workers (266-268) reported that caffeine or coffee increased serum free fatty acids (FFA) in dogs and man. The 1965 paper describes results in 34 fasting, normal male subjects, 18-63 years of age. Twelve were given instant coffee, containing 218 mg of caffeine, to which 150 mg sodium cyclamate and 15 mg saccharin were added. Venous blood samples were drawn before, and at 30-minute intervals after the beverage. For control values each subject drank an equal volume of hot water (500 ml) containing the same amounts of sweeteners, and blood samples were drawn as above. However, it is important to note that control and test runs were made on different days.

The results are interpreted as showing a significant ($P < 0.05$; Student's t test) increase in FFA after coffee compared to the FFA increase after water ; 67 % in the former and 20 % in the latter. A similar, but less marked, increase (46 %) in FFA followed intramuscular caffeine, 250 mg, in a separate group of 11 subjects.

Examination of the raw data (BELLET, *et al.*, 267, table 2) shows that FFA control levels are extremely variable in the test population as a whole, and also in the same subject on different days of testing (coffee *vs.* water). Some of the control values are so low as to raise doubt that subjects were fasting. The authors have focused on maximal FFA increments which they note to occur 120 to 180 minutes after coffee. However, this is not true of all subjects, and the tabulated data reveals five subjects in which it is unlikely that any real increase in FFA has occurred. Moreover, there is a suggestion of an early fall in FFA in some individuals, a point not mentioned by the authors. It is also known that serum FFA levels are extremely labile, and subject to alteration by a great many factors.

An F, or variance ratio, test on the control data in BELLET's table 2 (267) indicates that the 12 subjects are different populations on the coffee and on the water test days. Consequently, the t test is statistically invalid. Nevertheless, it appears that at least some of the subjects show real increments in FFA after coffee. This suggests that the FFA response to coffee or caffeine is, like other cardiovascular responses, one in which the normal population shows variation.

BELLET's work is of interest, as he states, because of the possible relationship between elevated FFA levels and subsequent development of coronary atherosclerosis. However, a newspaper account of his 1966 abstract stated that BELLET found the FFA increase to be much less marked when sugar was added to the coffee.

Two unusual reports on dietary sugar (YUDKIN 280 ; YUDKIN and RODDY 281) provide a link between the BELLET and PAUL studies. YUDKIN (280) shows that national sugar and fat intake in 41 countries are perfectly correlated. He concludes that « statistics relating fat intake to ischemic heart disease or diabetes mellitus in different populations may therefore express only as an indirect relationship, and the causal relationship may be with sugar ». YUDKIN and RODDY (281) then proceed to show that patients with occlusive atherosclerotic disease take more sugar in more cups of coffee or tea per day than do control patients. The difference, almost double the amount, is highly significant statistically ($P < 0.001$).

Possibly, therefore, sugar in coffee, rather than coffee *per se*, may be the real association in PAUL's study (277). If so, sugar's prevention or diminution of the coffee-induced rise in FFA means that elevation of serum FFA levels may not predispose to atherosclerosis. It suffices to say that any cause-effect relation between coffee-drinking and development of ischemic heart disease is at best tenuous.

SCHROEDER and his coworkers (282, 283) have observed that cadmium consumed in the diet tends to accumulate in the kidneys of man and animals and, in animals at least, appears to be associated with the production of hypertension. This effect of cadmium appears to be blocked if the zinc-cadmium ratio is high. There is some evidence that the zinc-cadmium ratio in human kidneys from hypertensive individuals is low. SCHROEDER has indicated that coffee and tea have a low zinc-cadmium ratio and may contribute to the cadmium load for man. It would appear from his data that only a very small percentage of man's daily cadmium intake would be contributed by coffee, but does indicate that coffee processing, in particular for instant coffees, should not include equipment which would introduce cadmium as an impurity.

CONCLUSIONS

It is clear that caffeine and coffee interact with the cardiovascular system in accordance with well-known pharmacologic principles. Foremost among these is the variability of response among individuals from a normal population. For example, some animals and men show a blood pressure rise, some a fall, and still others no effect, with the same dose of caffeine. A second principle, that the effect is proportional to dose, is also evident, though not strikingly so. The data suggest that the dose-effect curves for caffeine action on cardiovascular parameters are relatively flat. In other words, a small change in dose does not produce a large change in the effect. While this may indicate something about the intrinsic potency of caffeine, it is more likely a reflection of opposing actions of the drug within the cardiovascular system. Either way, the practical meaning is that the difference between one and two, or two and three, cups of coffee might not be readily detected in tests on the circulatory systems of most normal men. « Normal » men are those with no symptoms of cardiovascular disease, and accustomed to some regular intake of caffeine-containing beverages ; coffee, tea, coca-cola, etc.

A third pharmacologic principle is that some drugs induce a state of tolerance, in which the original effect diminishes or disappears upon repeated usage. This is so for caffeine. But we do not know how long it takes to acquire caffeine tolerance, or, once acquired, how long it takes to lose it. In sum, all the preceding principles probably underlie the fact that cardiovascular reactions to caffeine/coffee are negligible in the majority of the normal population.

Intolerant individuals generally show more marked cardiovascular reactions to caffeine-containing beverages. Within this group are some individuals considered « sensitive », and so they frequently describe themselves. Invariably aware of their state, they voluntarily avoid caffeine-containing beverages. That their number is not large may be inferred from the fact that in all the studies examined only two out of a great many subjects were truly « sensitive ».

The effects of caffeine-containing beverages may also be more marked, according to one study, in humans with hypertensive cardiovascular disease. But the issue is controversial. One should recall that hypertensive patients were included in many studies which showed no particularly distinctive reactions in these subjects. Factors other than reactivity, though, are important in evaluating caffeine effects on hypertensive patients. Thus, the cerebral vasoconstricting action may relieve their headaches, but could be viewed as a potentially dangerous type of reaction in hypertensives.

The preceding remarks, indeed almost all the studies of caffeine and coffee, relate to acute effects. Virtually nothing is known about chronic cardiovascular effects. Functionally, it appears that chronic abuse of coffee will provoke cardiac arrhythmias in a small fraction of the human population. Histopathological effects of chronic caffeine administration are uncertain in animals, and unknown in man, at least with respect to the cardiovascular system. Though the degree of coffee consumption may be one of many associative factors in some patients with ischemic heart disease, the interaction of the multiple factors is obscure. That is to say, the relevance of individual factors is not established. And it may be that sugar in coffee, rather than coffee *per se*, is the underlying factor. Cadmium (particularly when the Zn Cd intake ratio is low) has been reported to cause cardiovascular disease in animals and to be present in coffee. Present evidence indicates that only a very small percentage of man's total daily cadmium intake would be provided by coffee.

The matter of sugar in coffee illustrates a final point. Whereas the acute cardiovascular effects of coffee appear to depend largely on the caffeine content, we cannot say that the other components are inert. They have simply not been investigated. Nor can they be dismissed from consideration in terms of chronic effects of coffee-drinking. There is no *a priori* reason to assume that chronic effects of coffee-drinking, if such exist, are attributable to caffeine. Therefore it seems highly desirable to establish both the acute and chronic effects of coffee components other than caffeine.

REFERENCES

INTRODUCTION

1. GOODMAN, L. S. and A. GILMAN. — The Pharmacological Basis of Therapeutics, 3rd edition, The Macmillan Co., New York, 1965.

ANIMAL STUDIES

Heart rate and blood pressure

2. BUNAG, R. D. and E. J. WALASZEK. — Cardiovascular effects of substance P in the chicken. *Ann. N. Y. Acad. Sci.*, **104** : 437-48, 1963.

3. BUNIN, K. V. — Action of ephedrine, cordiamine and caffeine on the circulatory system in typhus. *Sovet. Med.*, **13** : 18-19, 1949.

4. CHARLIER, R. — Contributions à l'étude des propriétés tonicardiaques de la caféine, de la théobromine et de la théophylline. *Arch. Internat. Pharmacodynamie*, **62** : 370-76, 1939.

5. CRAVER, B. N. *et al.* — Kymographic registration of cerebrospinal fluid pressure (in dogs and cats) : effects of antihistaminics and common drugs. *Arch. Internat. Pharmacodynamie*, **80** : 310-19, 1949.

6. DIKSHIT, B. B. — The production of cardiac irregularities by excitation of the hypothalmic centers. *J. Physiol.*, **81** : 382-94, 1934.

7. ESVELD, L. W. and V. G. BYLSMA. — Influence of some medicaments on the irritability of the vasomotor center. *Arch. Neirland. Si. Exactes et Nat. Sci.*, **3** : C 14, 321-23, 1929.

8. HAHN, F. — Comparative studies of the action of various analeptics on convulsions and blood pressure. *Arch. Exptl. Path. Pharmakol.*, **198** : 491-508, 1941.

9. HAHN, F., D. PLESTER and W. RUMMEL. — Further investigations on the blood pressure effects of centrally acting analeptic substances. *Arch. Exptl. Path. Pharmakol.*, **211** : 223-34, 1950.

10. JUNKMANN, K. and W. STROSS. — Toxic stimulation of the vasomotor center, I. *Arch. Exptl. Path. Pharmakol.*, **131** : 1-17, 1928.

11. JUNKMANN, K. and W. STROSS. — Toxic stimulation of the vasomotor center, II. *Arch. Exptl. Path. Pharmakol.*, **131** : 18-24, 1928.

12. MARTINETTI, R. — Vasomotor effects of caffeine. *Arch. di Fisiol.*, **38** : 373-88, 1938.

13. MARTINETTI, R. — Azione inhibitrice di derivati purinici sull'impertensione arteriosa e sull' apnea da adrenalina. *Arch. di Fisiol.*, **38** : 389-96, 1938.

14. MIRANO, G. C. — L'azione della caffeina sulla pressione del pulso. *La Riforma Medica*, **21** : 38, 1906.

15. PEGEL, V. A., E. SILANT and K. A. LUKOVSKAYA. — Effect of different concentrations of caffeine and adrenaline on the cardiac and respiratory systems and the interrelation of these drugs in rabbits. *Trudy Tomsk. Univ.*, **123** : 139-59, 1953.

16. PILCHER, J. D. — The action of caffeine on the mammalian heart. *J. Pharmacol.*, **3** : 609-24, 1912.

17. SAFONOVA, A. D. — The effect of bromine, caffeine and several of their combinations on blood circulation and respiration with the intravenous administration of these preparations, Sbornik Trudox Omsk. Filiala Vsesoyuz. *Obshchestva Fiziologov, Biokhimikov i Farmakologov*, **1** : 297-301, 1958.

18. SALANT, W. — The effect of caffeine on the circulation. *J. Pharmacol.*, **3** : 468-69, 1912.

19. SCHMID, E., M. BUBNOFF and R. TAUGNER. — The effect of purine compounds on the circulation in the non-narcotized dog. *Arzneim. Forsch.*, **5** : 194-201, 1955.

20. SOKOLOVA, L. N. — Action of caffeine, camphor and corasol (metrazole) on the cardiovascular system of rabbits treated with diptheria toxin. *Farmakol. i Toksikol.*, **3** : 22-28, 1940.

21. SOLLMAN, T. and J. D. PILCHER. — Influence of caffeine on the mammalian circulation. I. Persistent effects of caffeine on the circulation. *J. Pharmacol.*, **3** : 19-48, 1912.

22. SOLLMAN, T. and J. D. PILCHER. — Influence of caffeine on the mammalian circulation. II. Influence of blood pressure on cardiac and vascular reactions. *J. Pharmacol.*, **3** : 48-51, 1912.

23. SOLLMAN, T. and J. D. PILCHER. — Influence of caffeine on the mammalian circulation. III. Influence of caffeine on blood pressure reactions. *J. Pharmacol.*, **3** : 51-64, 1912.

24. SOLLMAN, T. and J. D. PILCHER. — Acute effects of intravenous caffeine injections on the circulation. *J. Pharmacol.*, **3** : 66-93, 1912.

25. SPERANSKAJA-STEPANOVA, E. N. — The reactions of blood pressure to intravenous and intra-arterial injections. *Z. Ges. Exptl. Med.*, **88** : 616-21, 1933.

26. SWIRSKI, G. — Über die Beeinflussung des Vaguszentrums durch das Coffein. *Arch. für Gesamte Physiologie*, **104** : 260-93, 1904.

27. TANIGUCHI, K. — Pharmacological studies on the central vasoregulatory organization. *Yonago Igaku Zasshi*, **10** : 357-74, 1959.

28. TIFFENEAU, M. and A. BEAUNE. — Sympathomimetic action of caffeine. *Rev. Soc. Argent. de Biol.*, **10** : 525-29, 1934.

29. TSUBOI, S. — (Caffeine effect) on blood pressure. *Jap. J. M. Sc. IV, Pharmacol.*, **12** : 161-63, 1940.

30. VOLYNSKII, B. C. — Effects of caffeine on blood pressure in changing functional states of the body. *Farmakol. i Toksikol.*, **19** : 5-6, 1956.

31. WAELE, H. — Influence de l'alcoose et de l'acidose sur le mécanisme modérateur et accélérateur du cœur et sur l'action des substances vago- et sympathicomimétiques. *Comp. Rend. Soc. Biol.*, **94** : 1041-42, 1926.

32. WALTON, R. P. and O. J. BRODIE. — The effect of drugs on the contractile force of a section of the right ventricle under conditions of an intact circulation. Measurement of isometric systolic tension by means of calibrated springs attached to myocardiograph levers. *J. Pharmacol.*, **90** : 26-41, 1947.

33. YAMANOUTI, Y. — Über den pharmakologischen Wirkungsmechanismen von Kampfer und verwandten Onaleptica. *Fukuoka Acta Med.*, **31** : 1541-1620, 1938.

34. YAMANOUTI, Y. — Pharmacological action of camphor and related drugs. *Fukuoka Acta Med.*, **31** : 161-64, 1938.

35. ZHMURKIN, V. P., *et al.* — (On the effect of caffeine on blood circulation and regional vascular tonus). *Klin Med.*, **42** : 20-25, 1964.

Cardiac function

Isolated hearts

36. ARRIGO, L. — Impedographic equivalents of the activity of so-called membrane acting drugs on the isolated rabbit heart. *Arch. di Fisiol.*, **60** : [illegible] 1960 81-88, [illegible].

37. BALEHRADEK, J. — The effect of caffeine on the size of the temperature coefficient of heart rhythm. *Arch. Intern. Physiol.*, **35** : 1-8, 1932.

38. BRINLEY, F. J. — The effect of caffeine on the prevagus and postvagus Chick hearts. *Amer. J. Physiol.*, **100** : 357-61, 1932.

39. BRUNS, O. and H. ROSENCRANTZ. — Action of drugs on weakly contracting and overworked hearts. *Zeitschr. Ges. Exptl. Med.*, **49** : 430-45, 1926.

40. BÜLBRING, I. — Effect of some new substances with cardiac action on perfused frog hearts. *Arch. Exptl. Path. Pharmakol.*, **152** : 257-72, 1930.

41. CHOPRA, R. N., N. N. DAS and B. MUKERJI. — The action of certain cardiac drugs on embryonic heart explants. *Indian J. Med. Res.*, **26** : 271-78, 1938.

42. DAVID, I. — The action of caffeine on the work of the isolated frog heart. *Boll. Soc. Ital. Biol. Sper.*, **28** : 1977-79, 1952.

43. DE AGAZIO, A. — The action of histamine, insulin, ephetonine, caffeine and camphor on the isolated heart of Bufa vulfaris. *Atti Accad. Lincei*, **19** : 656-60, 1934.

44. EISMAYER, G. and H. QUINCKE. — Heart muscle tone. *Arch. Exptl. Path. Pharmakol.*, **137** : 362-79, 1928.

45. FLAUM, E. and R. RÖSSLER. — The cardiac action of the purine bodies. *Klin. Wochschr.*, **12** : 1489-91, 1933.

46. FUJII, M. — Pharmacological study of embryonal chicken hearts at different stages of development. I. *Folia Pharmacol. Japon.*, **4** : 309-30, 1927.

47. HARDMAN, H. F. — Molecular form of theophylline responsible for positive inotropic activity. *Circulation Res.*, **10** : 598-607, 1962.

48. KIESE, M. and H. GUMMEL. — The effect of caffeine on the heart. *Klin. Wochschr.*, **17** : 927-28, 1938.

49. KIKUCHI, I. — Pharmacologic study of the absolute pressure of the heart. I. Study of the frog heart. *Tohoku J. Exptl. Med.*, **11** : 116-41, 1928.

50. KIKUCHI, I. — Pharmacologic study of the absolute pressure of the heart. II. Study of the rabbit heart. *Tohoku J. Exptl. Med.*, **11** : 293-307, 1928.

51. KÖNIG, W. — Herzarbeit ohne Sauerstoff. III. Mitteilung. *Arch. Exptl. Path. Pharmakol.*, **127** : 346-65, 1928.

52. KUKOVETZ, W. R., N. HAUGAARD, M. E. HESS and J. SHANFELD. — Relation between contraction and phosphorylase activity of the rat heart. *Arch. Exptl. Path. Pharmakol.*, **238** : 119-21, 1960.

53. KUSCHINSKY, G. and E. MUSCHOLL. — Action of purine substances on cardiac contraction force. *Klin. Wochenschr.*, **34** : 895-96, 1956.

54. KUSCHINSKY, G. and E. MUSCHOLL. — Effect of theophylline, caffeine and theobromine on contractility, sensitivity, refractory time, and spontaneous frequency of the isolated heart muscle in the cat. *Arch. Exptl. Path. Pharmakol.*, **229** : 348-59, 1956.

[illegible] USCHEN[illegible] A. A.

[illegible] LÜBUSCGL[illegible], A. A. — Influence of the [illegible] reaction of certain poisons. *Zhurnal Exptl Biol. Med.*, 10 : 277-83, 1929.

56. LÜLLMAN, H. — The effect of caffeine and theophylline on the heart muscle. *Arch. Kreislauffforsch.*, **23** : 12-76, 1955.

57. McCARTNEY, E. and F. RANSOM. — Sodium citrate and the action of certain drugs upon the frog heart. *J. Physiol.*, **51** : 287-93, 1917.

58. MATANO, M. — Effects of anaphylaxis and various drugs on the potassium contraction of the atria in guinea pigs. *Nagasaki Med. J.*, **39** : 486-95, 1964.

59. NAYLER, W. G. — Effect of caffeine on cardiac contractile force, activity and radiocalcium movement. *Amer. J. Physiol.*, **204** : 969-74, 1963.

60. NEUROTH, G., K. WEZLER and G. SEIGLITZ. — Action of purine compounds and their substituents on the dynamics of the isolated spontaneously beating frog heart. *Arch. Exptl. Path. Pharmakol.*, **220** : 378-90, 1953.

61. NIIMOTO, I. — The combined cardiac effects of odorin and quinine, metrazole and caffeine. *Mitt. Med. Ges. Okayama*, **51** : 2231, 1939.

62. OELSSNER, W. — Dye permeability in isolated beating frog hearts. *Arch. Exptl. Path. Pharmakol.*, **212** : 195-204, 1951.

63. OSWALD, L. — Über die Beeinflussung des Koronarkreislaufs durch Gifte. *Arch. Exptl. Path. Pharmakol.*, **51** : 64-83, 1904.

64. PELTOLA, P. and A. VARTIAINEN. — Action of antipyrine on the isolated frog heart. *Acta Pharmacol. Toxicol.*, **3** : 219-24, 1947.

65. PIETRO, C. — Action of caffeine on the isolated frog heart. I. Spreading of diastolic action on the isolated toad heart by very small doses of caffeine : the so-called caffeine benzoate and sodium obstruct the action of pure caffeine. *Arch. Farm. Sper.*, **81** : 137-49, 1953.

66. PIETRO, C. — Research on the action of caffeine. II. The mechanism of action of caffeine on the isolated frog heart. *Arch. Farm. Sper.*, **82** : 58-66, 1954.

67. SANDERS, R. — Dynamics of the frog heart : action of strophanthin, caffeine, camphor and cardiazole. *Arch. Exptl. Path. Pharmakol.*, **125** : 358-80, 1927.

68. SASAKI, M. — (Caffeine effects) on auriculoventricular conduction of toad's heart : perfusion experiments. *Jap. J. M. Sc. III. Biophysics*. **4** : 88-90, 1938.

69. SIMON, I. and C. PIETRO. — Action of caffeine on the isolated frog heart. *Boll. Soc. Ital. Biol. Sper.*, **29** : 310-11, 1953.

70. STAUB, H. and W. GRASSMAN. — The minimum effective dose of some toxins on isolated mammalian hearts (with contributions as to method). *Arch. Exptl. Path. Pharmakol.*, **154** : 317-341, 1930.

71. SUSANNA, V. — Action of caffeine upon the frequency of heart beat. *Arch. Intern. Pharmacodynamie*, **27** : 265-75, 1922.

72. TAKABE, T. — Dynamic studies of the action of cardiotonics on frog hearts. *Jap. J. M. Sc. IV. Pharmacol., S. Proc. Jap. Pharmacol. Soc.*, **4** : 138-41, 1934.

73. TAKABE, T. — Dynamische Studien über Kardiotonika am Froschherzen. *Mitteil Med. Ges.* Tokio, **49** : 510-44, 1935.

74. TSUDA, Y. — Pharmacological investigation of heart strip preparations according to Loewe. I. Influence of drugs on the release of automaticity and the pulse. *Folia Jap. Pharmacol.*, **3** : 456-77, 1926.

75. ZAKRIVIDOROGA, S. P. — Comparative influence and therapeutic range of action of some drugs on isolated hearts of cold-blooded animals (frogs). *Farmakol. i Toksikol.*, **8** : 10-14, 1945.

Hearts of intact animals

76. BARRY, D. T. — Influence of certain drugs on the work done by the heart on conditions of experimental mitral lesion. *Arch. Internat. Pharmacodynamie*, **38** : 111-21, 1930.

77. BOCK, J. — Über die Wirkung des Coffeins und des Theobromins auf das Herz. *Arch. Exptl. Path. Pharmakol.*, **53** : 367-99, 1900.

78. BRINLEY, F. J. — The effect of caffeine upon the cardiac rhythm of adult fish hearts. *Physiol. Zool.*, **7** : 400-7, 1934.

79. CHAUCHARD, P. — Changes in the excitability of the cardioinhibitor and cardioaccelerator nerves of the dog under the influence of various types of drugs which modify the cardiac rhythm. *Compt. Rend. Soc. Biol.*, **128** : 50-53, 1938.

80. CHAUCHARD, P., *et al.* — Influence of sugars on excitation of the heart by caffeine. *Compt. Rend. Soc. Biol.*, **139** : 394-95, 1939.

81. CUSHNEY, A. R. — On the action of caffeine on the mammalian heart. *Arch. Internat. Pharmacodynamie*, **9** : 169-80, 1901.

82. FREDERICQ, H. — The excitation of the cardiac vagus and its modification under the influence of caffeine. *Arch. Intern. Physiol.*, **13** : 107-14, 1913.

83. FREDERICQ, H. and Z. M. BACQ. — Caffeine effect on cardiac vagus of turtle. *Compt. Rend. Soc. Biol.*, **124** : 269-71, 1937.

84. FREDERICQ, H. and Z. M. BACQ. — On cardiac vagus of turtle. *Compt. Rend. Soc. Biol.*, **124** : 1256-57, 1937.

85. HASKELL, C. C. — The action of caffeine on the poisoned heart. *J. Amer. Pharm. Assoc.*, **15** : 744-47, 1926.

86. HUKUDA, T. — The central origin of the cardiotonic action. *Hukuoka Acta Med.*, **32** : 86-87, 1939.

87. JOURDAN, F., G. FAUCON and G. SCHAFF. — Cardiac stimulating action of some vasodilator agents. *Compt. Rend. Soc. Biol.*, **156** : 1855-59, 1962.

88. KIESE, M. and R. S. CARAN. — Mechanical work, size and oxygen consumption of the warm-blooded heart. *Klin. Wochschr.*, **16** : 1219-20, 1937.

89. PILCHER, C., C. P. WILSON and T. R. HARRISON. — The action of drugs on cardiac output. III. The effect of caffeine sodiobenzoate on the cardiac output of dogs. *Amer. Heart J.*, **2** : 618-30, 1927.

90. REGGIANI, G. — Action on the ergograph of antipyrine and caffeine. *Bull. Scienze Med.*, **98** : 289-312, 1926.

91. ROTH, G. B. — Cardiac effects of acetanilide, caffeine and its citrate. *J. Pharmacol.*, **27** : 249, 1926.

92. SAITO, C. — The alteration of the electrical excitability of the heart vagus by various means. I. The action of heart remedies. *Acta Schol. Med. Univ. Imp. Kioto*, **16** : 145-51, 1933.

93. SALANT, W. and J. E. NADLER. — The relation between cardiac reactions to drugs and the hydrogen-ion concentration of the blood. *Amer. J. Physiol.*, **78** : 308-21, 1926.

94. SCHAFF, G. and G. FAUCON. — Comparison of the action of vasodilators on the contractions of the myocardium (in dogs). *Arch. Intern. Pharmacodynamie*, **145** : 213-32, 1963.

95. SUBBOTIN, P. M. — Specific activity of cardiac glucoside preparations in their most commonly prescribed combinations with other drugs. *Farmakol. i. Toksikol.*, **3** : 13-19, 1940.

96. Van EGMOND, A. — Action of several drugs in complete heart block. *Arch. Ges. Physiol.*, **154** : 140-62, 1914.

97. WATANABE, T. — Effect of various drugs on the refractory phase of heart muscle, I-IV and Supplement. *Zyuzenkai Zassi*, **45** : 2947-57, 1940.

Arrhythmias

98. BEATTIE, J., G. BROW and C. LONG. — The vegetative nervous system. The Williams and Wilkins Co., Baltimore, p. 249, 1930.

99. BENTHE, H. F. — Dromotropic effect of caffeine on the heart muscle of the frog explained as sensitization to potassium. *Arch. Exptl. Path. Pharmakol.*, **223** : 285-93, 1954.

100. BOSE, B. C., R. VIJAJVARGIYA, A. W. BHAGWAT and N. M. ROY. — Effects of purines and ATP (ase, osine, triphosphate) on heart. *Current Sci.* (India), **33** : 526-27, 1964.

101. CHENEY, R. H. — Cardiac automaticity effects of caffeine and nicotine. I. Caffeine influence upon the response of the sinoauricular strip. *J. Pharmacol*, **54** : 213-21, 1935.

102. DAUTREBANDE, L. and R. CHARLIER. — Le nitrite d'anyle et la caféine facteurs empêchants de la syncope adrénalino-chloroformique et de la syncope adrénalino-benzolique. *Arch. Intern. Pharmacodynamie*, **66** : 29-40, 1941.

[illegible]ARETT, T. and W. SLEATOR. — Effects of [illegible] on mammalian atrial muscle, and its [illegible]action with adenosine and calcium. *J. Phar[illegible]*, **148** : 202-14, 1965.

[illegible]VA, N. M. — Reaction of the organism to [illegible] glycosides on stimulation of the central [illegible] system. Fisiol. i. Patol. Serdechno-[illegible]tsistoi Sistemy v Klin i Ekspt., Sbornik [illegible] 227-37, 1958.

[illegible] Action of toad poison and strophanthin [illegible] the refractive phase of isolated frog heart. [illegible], **86** : 427-46, 1927.

[illegible] H. B. and J. D. WOODLEY. — Effect of [illegible] and theobromine upon digitalis toxicity ; [illegible]mental study. *J. Pharmacol.*, **33** : 465-73, [illegible].

[illegible]ONI, P. — Pharmacological basis of thera[illegible]tic combinations of cardiac remedies. *Rass. [illegible]-Sci.* (Inst. Biochim. Ital.), **17** : 531-37, 1939.

[illegible]ASALMA, G. — Pathogenic points on expe[illegible]ental arrhythmia from caffeine-adrenaline. [illegible] *Soc. Ital. Sper.*, **32** : 1637-39, 1956.

[illegible] A. — Modifying effects of caffeine on action [illegible] acetylcholine on heart of tortoise and striped [illegible]scle of frog. *Arch. Internat. Physiol.*, **44** : [illegible]-94, 1937.

[illegible]SRAZHENSKII, A. M. — The tonic action of [illegible]affeine on the frog heart *in situ*. *Z. Ges. Exptl. [illegible]*, **71** : 49-71, 1930.

[illegible] L., R. MUSCHAWECK, and J. HERGOTT. — [illegible]tagonism between adenosine and methyl[illegible]anthines on the stimulus conductivity of the [illegible]eart. *Arch. Exptl. Path. Pharmakol.*, **231** : 586-[illegible] 1957.

[illegible] I., R. W. LOEHNING and H. UEYAMA. — [illegible]elation between sympathomimetic amines and [illegible]methylxanthines inducing cardiac arrhythmias. *[illegible]esthesiology*, **22** : 926-32, 1961.

[illegible]thological changes

[illegible]KE, W., *et al.* — An electrocardiographic study [illegible] experimental heart disease. *Amer. Heart J.*, **2** [illegible] 302-10, 1927.

[illegible]EY, R. H. — Myofibrillar modifications indu[illegible]ed by caffeine in cardiac muscle of the frog. [illegible] *Cell. Comp. Physiol.*, **18** : 15-20, 1941.

[illegible]CHRA-LYSANOWICZ, Z., M. CORSKI and [illegible] KEDRA. — Effect of nicotine and caffeine on [illegible]evelopment of arteriosclerosis in rabbits. [illegible]n. *Marie Curie-Sklodowska, Lublin-Polonia* [illegible] **D14** : 181-206, 1961.

[illegible]CHMER, W. — Observations on the vaso[illegible]dilatic effect of caffeine ; the origin of idio[illegible]atic cardiac hypertrophy. *Deut. Arch. Klin. [illegible]*, **180** : 318-26, 1937.

[illegible]OVIRIA, E. M. — Histopathology of the nervous [illegible]ements in the heart and the aorta of dogs [illegible] intravenous injection of adrenaline and [illegible]ffeine. *Bull. Eksp. Biol. Med.* (Eng.), **52** : 967-[illegible] 1962.

[illegible] A. and G. CABRINI. — The survival and the [illegible]ological changes of the heart through the [illegible] of caffeine. *Boll. Soc. Ital. Sper.*, **6** : 594-96, [illegible].

119. MEHES, G., L. SZEKERES, J. KOVACSICS and F. VANGA. — Heart injury caused by caffeine after single and chronic administration. *Acta Physiol. Hung.*, **6** : 113-21, 1954.

120. MILERE, M. and D. IONCHEV. — Effect on various drugs on electrocardiographic tracing in hypoxia. *Suvaem Med. Sofia.*, **5** : 47-54, 1954.

121. MROZIKIEWICZ, A., A. MAZUROWA and W. KAZIMIERZ. — Electrocardiographic and histological changes in the heart muscle of rabbits after glutamic acid administration. *Patol. Polska*, **15** : 327-38, 1964.

122. SERANOVA, A. I. — Experimental study of the effect of certain neurotropic pharmacological preparations on development of hypercholesterolemia and atherosclerosis. I. Effect of preparations stimulating the central nervous system ; caffeine and phenamine. *Ezhegodnik Inst. Eksperim. Med., Akad. Med. Nauk SSSR*, Leningrad, **7-8** : 220-23, 1963.

123. SHKHVATSABAYA, I. K. — The effect of some neurotropic substances upon the development of experimental atherosclerosis. *Biull. Eksptl. Biol. i. Med.*, **41** : 39-42, 1956.

124. WENZEL, D. G., J. D. MacCARTHY and C. O. RUTLEDGE. — Evaluation of caffeine and ouabain as permissive agents in the production of myocardial necrosis. *J. Pharm. Sci.*, **53** : 567 68, 1964.

Effects on specific vascular beds

Peripheral, visceral, pulmonary

125. ANSCHUTZ, H. F. and W. SCHROEDER. — Effect of various pharmacological substances on the circulation in the capillaries and the arteriovenous anastomoses in the extremities of dogs. *Z. Ges. Exptl. Med.*, **116** : 291-99, 1950.

126. BARKSDALE, I. S. — The effects of certain drugs on the capillaries : atropine, eserine, sparteine, digitalis, caffeine, and cucurbocitrin. *Southern Med. and Surg.*, **89** : 27-32, 1927.

127. BECQ, L. and L. PLUMIER. — Action cardiovasculaire de quelques dérivés xanthiques. *J. de Physiologie et Pathologie Générale*, **8** : 10-21, 1906.

128. BOCK, H. E. — The vascular effect of strophanthin, theocin and caffeine. Studies on the surviving extremity and lung (of dogs). *Arch. Exptl. Path. Pharmakol.*, **166** : 634-48, 1932.

129. BURGI, S. — Acceleration, inhibition and disorganization of spontaneous rhythmic arterial contractions. *Helv. Physiol. Pharmacol. Acta*, **3** : 215-29, 1945.

130. BURGISON, R. M., W. E. O'MALLEY, C. K. HEISSE and J. C. KRANTZ, Jr. — Pharmacologic studies with 8-(para-aminobenzyl)-caffeine and certain related compounds. *J. Pharmacol.*, **119** : 107-13, 1957.

131. EBBECKE, U. and A. JAGER. — *Pfluger's Arch. Ges. Physiol.*, **232** : 36-41, 1933.

132. FUJIMORI, K. — The reaction of blood vessels. II. The effect of several nerve and muscle poisons. *Acta Schol. Med. Univ. Imp.*, Kioto, **15** : 126-27, 1932.

133. HATTA, H. and O. KENICHI. — Contractibility of the lymphatic vessels of dogs. *Hiroshima J. Med. Sci.*, **2** : 319-22, 1954.

134. IMAI, H. — The action of various substances on the vessels of the isolated perfused bone marrow. *Folia Endocrinol. Japon.*, **12** : 58, 1936.

135. ITO, N — Influence of drugs on electrical stimulation of the nervous auricularis magnus. *Folia Jap. Pharmacol.*, **3** : 27-53, 1926.

136. ITO, N. — Effect of caffeine sodiobenzoate on blood vessels of lungs, *Jap. J. M. Sci., IV, Pharmacol.*, **9** : 76-78, 1936.

137. ITO, N. — The action of sodium caffeine benzoate on the lung blood vessel. *Nagoya Igakkai Zasshi*, **45** : 1, 1937.

138. JOURDAN, F. and G. FAUCON. — Ephemeral character of the effect of vasodilators administered intra-arterially. *Therapie*, **14** : 844-53, 1959.

139. KADZIELA, W. and Z. BARGIEL. — Vasomotor reactions of the vascular network of the rabbit ear during acute fatigue under the influence of certain neurovasotropic substances. *J. Physiol.* (Paris), **55** : 193-94, 1963.

140. KOIZUMI, Z. — The effect of organic acid derivative of purine bases on the blood vessels of surviving hind legs of frog, *Sei-i-Kai Med. J.*, **55** : 2607-24, 1936.

141. KONOVALOV, P. F. — Reflex effect of cardiovascular drugs arising from the venous receptors of the frog. *Bull. Exptl. Biol. Med.* (USSR), **45** : 209-13, 1958.

142. KÜSTNER, H. and H. SIEDENTOPF. — Untersuchungen über die Biologie und Pharmakologie der Plazenta. III. Mitteilung. Reaktionsfähigkeit der Plazentagefässe auf pharmakologische Mittel. *Arch. Gynakol.*, **140** : 298-312, 1930.

143. LANGENDORF, H., H. G. HOHMANN and R. K. ZAHN. — Reaction of individual capillaries in frogs' webs under influence of drugs with vasomotor activity, recorded with an objective method of registration. *Z. Ges. Exptl. Med.*, **122** : 178-98, 1953.

144. MALOFF, G. and C. WAINSTEIN. — Pharmakologische Untersuche an isolierten Froschvenen. *Pfluger's Arch. Ges. Physiol.*, **229** : 33-37, 1931.

145. MICHALEVSKAYA, P. V. — Action of poison on the pulmonary blood vessels and the effect of pressure upon the vasomotor reaction. *Z. Ges. Exptl. Med.*, **71** : 489-95, 1930.

146. MISLIN, H. and D. RATHENOW. — Modification of the spontaneous rhythm of isolated mesenteric lymph vessels of *Cavia porcellus* by various drugs. *Helv. Physiol. Pharmacol. Acta*, **19** : C87-C90, 1961.

147. OCHIAI, A. — The pharmacological reactions of arteries, veins and capillaries, I., *Folia Pharmacol. Japon.*, **7** : 19-20, 1928.

148. OKADA, M. — The action of some drugs upon the arterioles and capillaries. *Okayama-Igakkai-Zasshi*, **40** : 205-14, 1928.

149. PLESTER, D. — Mechanism of action of procaine and procaine-caffeine (impletol) in vascular disturbances. *Klin. Wochschr.*, **29** : 391, 1951.

150. PREOBRAZHENSKII, A. M. — Materials for the comparative pharmacology of vascular systems ; effect of poisons on the vessels of the ear in sheep. *Arch. Exptl. Path. Pharmakol.*, **122** : 198-206, 1928.

151. QUIMBY, C. W., *et al.* — Effects of aminophylline and other xanthines on the pulmonary circulation. *J. Pharmacol.*, **122** : 396-405, 1958.

152. SAKAMOTO, M. — The action of vasoconstrictors and vasodilators on the vessels of the rabbit testis. *Folia Endocrinol. Japon.*, **11** : 40-41, 1935.

153. TERASAKI, M. — Pharmacological contribution to mesenteric circulation in comparison with cerebral circulation. *Nippon Yakurigaku Zasshi*, **55** : 78-91, 1959.

154. TROFIMOVA, Z. G. — Venous blood circulation in dogs under action of substances which alter the level of arterial pressure. *Doklady Akad. Nauk. SSSR*, **111** : 232-35, 1957.

155. VALEEVA, Z. T. — Effects of some drugs on lymph ducts. *Farmakol. i. Toksikol.*, **23** : 258-64, 1960.

156. YOSHIYUKI, K. — Electric stimulation of the blood vessels. *Acta Schol. Med. Imp.*, Kioto, **17** : 22-26, 1934.

Cerebral

157. BEREZIN, V. I. — Action of poisons on the vessels of the brain. *Russky Vrstch*, **15** : 513, 1913.

158. BIRZIS, L., *et al.* — The action of stimulating and depressing drugs on local cerebral impedance and circulation. *Psychopharmacologia*, **6** : 256-66, 1964.

159. BOUCKAERT, J. J. and F. JOURDAN. — Action of caffeine, papaverine and barium chloride on the cerebral blood vessels. *Compt. Rend. Soc. Biol.*, **120** : 257-59, 1935.

160. BOUCKAERT, J. J. and F. JOURDAN. — The physiology and pharmacology of the cerebral vessels. IV. The action of adrenaline. *Arch. Intern. Pharmacodynamie*, **54** : 109-28, 1936.

161. DUMKE, P. R. and C. F. SCHMIDT. — Quantitative measurements of cerebral blood flow in the macoque monkey. *Amer. J. Physiol.*, **138** : 421 31, 1943.

162. FINESENGER, J. E. — Effect of caffeine on cerebral vessels. The vagal pathway of the vasodilator impulses. *Arch. Neurol. and Psychiatr.*, **28** : 1290-1325, 1932.

163. GOTTSTEIN, U., W. NIEDERMAYER and A. BERNSMEIER. — The cerebral circulation under the influence of vasoactive substances ; animal experiments with Euphyllin, Cordain, caffeine, Roniacol, papaverine and Hydergine. *Z. Ges. Exptl. Med.*, **131** : 430-39, 1959.

164. KUHN, J. — Action of medicinal substances and their combinations upon the intracranial vessels. *Arch. Exptl. Path. Pharmakol.*, **94** : 74-96, 1922.

165. NOELL, W. — The action of theophylline and other drugs on the blood circulation in the brain. *Z. Ges. Exptl. Med.*, **110** : 589-600, 1942.

166. NORCROSS, N. C. — Intracerebral blood flow. An experimental study. *Arch. Neurol. Psychiatr.*, **40** : 291-299, 1938.

167. POPESCU, M. — The vascular action of caffeine : the mechanism and the sensitivity of the cerebral vessels to caffeine. Acad. Rep. Populare Romine Baza Cercetari Stiint. Ti, Isoara. *Studii Cercetari Stiint., Ser. Stiinte Med.*, **3** : 47-55, 1955.

168. SAISHO, A. — Cerebrospinal fluid pressure and cerebral circulation as related to headache and vomiting. II. *Folia Pharmacol. Japon.*, **48** : 133-40, 1952.

169. SCHMIDT, C. F. and J. HENDRIX. — The action of chemical substances on cerebral blood vessels. *Proc. Soc. Res. Nerv. Ment. Dis.*, **18** : 229-76, 1937.

170. SCHNEIDER, M. — The regulation of blood flow through the brain. I., *Arch. Exptl. Path. Pharmakol.*, **175** : 606-39, 1934.

171. SOKOLOFF, L. — The action of drugs on the cerebral circulation. *Pharm. Rev.*, **11** : 1-85, 1959.

Coronary

172. BODO, R. — The effect of the « heart tonics » and other drugs upon the heart tone and coronary circulation. *J. Physiol.*, **64** : 365-87, 1928.

173. DATE, Y. — The action of certain drugs on the coronary flow of the dog. *Igaku Kenkyu*, **28** : 885-95, 1958.

174. HEATHCOTE, R. St. A. — The action of caffeine, theobromine and theophylline on the mammalian and betrachian heart. *J. Pharmacol.*, **16** : 327-44, 1921.

175. HEIMANN, W. and W. WILBRANDT. — Untersuchungen über coronardilatierende Wirkungen am Langendorff-Präparat und ihre Deutung auf der Grundlage eines Verdrängungs-Antagonismus. *Helv. Physiol. et Pharmacol. Acta*, **12** : 230-41, 1954.

176. IHASHI, S. — An age factor in susceptibility of the heart and coronary vessel of rabbits to heart stimulants. *Sei-i-Kai Med. J.*, **56** : 909-27, 1937.

177. IWAI, M. and K. SASSA. — Modification of the coronary circulation by purine derivatives. *Arch. Exptl. Path. Pharmakol.*, **99** : 215-25, 1923.

178. JOURDAN, F. and G. FAUCON. — Xanthine derivatives and coronary dilatation. *Arch. Internat. Pharmacodynamie*, **116** : 423-49, 1958.

179. LINDEN, E. and L. N. KATZ. — Further observations on the action of drugs on the caliber of coronary vessels. Papaverine hydrochloride, digitalis derivatives, aminophylline, caffeine, glucose, calcium gluconate, and metrazole. *J. Pharmacol.*, **72** : 306-10, 1941.

180. LOCHNER, W., H. MERCKER and E. SCHURMEYER. — Effect of vasomotor active substances on the oxygen saturation of the blood in the coronary sinus. *Arch. Exptl. Path. Pharmakol.*, **227** : 373-82, 1956.

181. LOCHNER, W. and H. HIRCHE. — Stimulatory action of some substances on the chemical receptors of the carotid sinus and on the dilation of the coronary artery. *Naturwissenschaften*, **49** : 259, 1962.

182. MALORNY, G. — New ergotamine and calcium effect on the Langendorff heart. *Arch. Exptl. Path. Pharmakol.*, **225** : 139-42, 1955.

183. MUSCHAWECK, R., W. PFAFF and J. HERGOTT. — Effect of purine compounds on the coronary-constricting effect of potassium chloride and cardiac glycosides. *Arch. Exptl. Path. Pharmakol.*, **236** : 226-29, 1959.

184. NUKI, B. — Pharmacology of the coronary circulation. *Sogo Igaku*, **14** : 1056-65, 1957.

185. OETTEL, H. — The dynamics of coronary dilating substances. *Z. Ges. Exptl. Med.*, **113** : 181-93, 1943.

186. OKI, T. — The action of xanthine derivatives on respiration, blood pressure and coronary blood vessels. *Igaku Kenkyu*, **28** : 2017-30, 1958.

187. RABE. — The reaction of coronary vessels to drugs. *Zeitschrift für Experimentelle Pathologie*, **11** : 175, 1912.

188. ROZIN, D. G. — Mechanism of action by antiffeine on cardiac coronary vessels. *Farmakol. i. Toksikol.*, **27** : 232-35, 1954.

189. SAKAI, S. and S. SANEYOSHI. — The action of several heart drugs (strophanthin, caffeine, diuretin) upon the coronary vessels. *Arch. Exptl. Path. Pharmakol.*, **78** : 331-46, 1915.

190. SEGRE, G. — Coronary dilation and constriction induced by methylxanthines with respect to sodium nitrite. *Arch. Ital. Sci. Farmacol.*, **9** : 369-88, 1959.

191. SMITH, F. M. — The coronary circulation. *Arch. Internal Med.*, **40** : 281-91, 1927.

Coffee studies in animals

192. BALTACEANO, G. and C. VASILIU. — Action of coffee on arterial pressure. *Compt. Rend. Soc. Biol.*, **113** : 1247-49, 1933.

193. BUSQUET, H. and M. TIFFENEAU. — The role of caffeine in the cardiac action of coffee. *Compt. Rend. Soc. Biol.*, **155** : 362-65, 1912.

194. CAMPOS, F. and D. De OLIVEIRA. — Acção do infuso de café sobre o sistema vascular. *Brasil-Med.*, **46** : 241-43, 1932.

195. PACHON, V. and E. PERROT. — Sur l'action cardiovasculaire du café vert, comparée à celle des doses correspondantes de caféine. *Compt. Rend. Soc. Biol.*, **150** : 1703-5, 1910.

STUDIES IN MAN

Blood pressure and heart rate

Caffeine

196. BAICHENKO, I. P. and G. E. VLADIMIROV. — The blood supply of the tissues of the organism at high altitudes and its changes after caffeine administration. *Klin. Med.* (USSR), **17** : (11) 99-112, 1939.

197. BÖGER, A., B. DEPPE and K. WEZLER. — Die Dynamik des menschlichen Kreislaufes unter der Wirkung von Kreislaufmitteln. I. Typische Reaktionsablauf. *Arch. Exptl. Path. Pharmakol.*, **189** : 480-508, 1938.

198. BRANDT, F. — Die Abhängigkeit des Venedruckes von der Grösse der zirkulierenden Blutmenge, zugleich ein Beitrag zu seiner klinischen Bedeutung. *Zeitschr. Klin. Med.*, **116** : 398-448, 1931.

199. BUSSE, H. — Effect of some drugs affecting the circulation on the rhythmic blood pressure fluctuations in men. *Z. Kreislaufforsch.*, **36** : 233-46, 1944.

200. DENKER, P. G. — The effect of caffeine on the cerebrospinal fluid pressure. *Amer. J. Med. Sci.*, **181** : 675-81, 1931.

201. FUCHS, E. — Action of caffeine on the blood pressure of healthy individuals. *Med. Monatssch.*, **2** : 46-50, 1948.

202. GIBBS, F. A., E. S. GIBBS and W. G. LENNOX. — The cerebral blood flow in man as influenced by adrenaline, caffeine, amyl nitrite and histamine. *Amer. Heart J.*, **10** : 916-24, 1935.

203. GILMAN, A. — Caffeine and health. II. *Hygea*, **13** : 878, 1935.

204. GROLLMAN, A. — The action of alcohol, caffeine and tobacco on the cardiac output (and its related functions) of normal men. *J. Pharmacol.*, **39** : 313-29, 1930.

205. KLEPZIG, H., *et al.* — Circulatory changes during and after exercise following administration of energizing substances. *Z. Klin. Med.*, **155** : 506-12, 1959.

206. LOMAN, J. and A. MYERSON. — The action of certain drugs on the cerebrospinal fluid and on the internal jugular venous and systemic arterial pressures of man. *Arch. Neurol. and Psychiatr.*, **27** : 1244, 1932.

207. LUCAS, W. P. — Cardiac stimulants : strychnine and caffeine. *Amer. J. Diseases of Children*, **7** : 208-29, 1914.

208. MAKINO, K. — Effects of various vasodilator drugs on cerebral circulation. *J. Jap. Soc. Int. Med.*, **47** : 768-79, 1958.

209. MARBACH, G., G. SCHAFF and M. T. SCHWARTZ. — Effects of alcohol and caffeine on cardiac frequency and respiratory frequency during sleep, *Compt. Rend. Soc. Biol.*, **156** : 1522-25, 1962.

210. MARTINETTI, R. — Modificazioni pressorie immediate all iniezione endovenosa di caffeina nell uomo. *Arch. Farmacol. Sperim e Sci. Aff.*, **66** : 59-68, 1938.

211. MARTINETTI, R. and A. FORCONI. — Action of caffeine in healthy or hypertensive subjects. *Policlinico (Med.) Sect.*, **44** : 286-98, 1937.

212. MEANS, J. H. and L. H. NEWBURGH. — The effect of caffeine upon the blood flow in normal human subjects. *J. Pharmacol.*, **7** : 449-65, 1915.

213. MOYER, J. H., *et al.* — The effect of theophylline with ethylene-diamine (amino-phylline) and caffeine on cerebral hemodynamics and cerebrospinal pressure in patients with hypertensive headaches. *Amer. J. Med. Sci.*, **224** : 377-85, 1952.

214. PELLEGRINI, M. — The antagonism between caffeine and adrenaline. Experiments on man. *Sperimentale*, **93** : 549-57, 1939.

215. PROKOP, L. — Altering the effect of adrenaline with caffeine. *Med. Klin.* (Munich), **59** : 2016-20, 1964.

216. PROPP, M. V. — Effect of small doses of bromide and caffeine on the vascular reflexes in man during ageing period. *Zhur. Vysshei Nervnoi Deyatel Nosti Im. I. P. Pavlova*, **9** : 191-95, 1959.

217. SEBOK, I. — Effects of caffeine in hypertension. *Deutsche Med. Wschr.*, **75** : 1067-70, 1950.

218. SCHWEISHEIMER, W. — Effect of caffeine on the heart. *Tea and Coffee Trade J.*, **111** : 12, 1956.

219. SHENKIN, H. A. — Effects of various drugs upon cerebral circulation and metabolism in man. *J. Appl. Physiol.*, **3** : 465-67, 1951.

220. STARR, I., C. GAMBLE, A. MARGOLIES, D. DONAL, Jr, N. JOSEPH and E. PARK. — A clinical study of the action of 10 commonly used drugs on cardiac output, work and size, on respiration, on metabolic rate, and on the electrocardiogram. *J. Clin. Invest.*, **16** : 799-823, 1937.

221. WOOD, H. C. — The effects of caffeine on the circulatory and muscular systems. *Therapeutic Gazette*, **36** : 6-13, 1912.

Coffee and caffeine

222. ARCHANGELSKY, C. T. — Die Wirkung des Destillats von Kaffee und von Tee auf Atmung und Herz. *Arch. Internatl. Pharmakodynamie*, **7** : 405-24, 1900.

223. BINZ, C. — Die Wirkung des Destillats von Kaffee und Tee auf Atmung und Herz. *Zentralblatt für innere Medizin*, **21** : 1169-76, 1900.

224. BUHR, G. — The action of caffeine and coffee on the heart and circulation in man. *Arztl. Forsch.*, **12** : 584-90, 1958.

225. BUHR, G. — Modification by condensed milk or a protein-valerian-polyphosphate complex of the effect of caffeine on the circulation in man. *Med. Ernahrung.*, **4** : 118-22, 1963.

226. CASSINIS, U. — Azione del caffè della caffeina, del cloruro di sodio nello sforzo degli atleti. *La Ric Sci.*, **1** : 353, 1933.

227. DALLA TORRE, L. and R. BOLDRINI. — Effect of natural coffee and decaffeinated coffee on cardiovascular system. *Minerva Med.*, **1** : 853-56, 1952.

228. FLEISCH, A. and V. WENNER. — Les effets physiologiques du café et des extraits de café. *Schweiz. Med. Wochenschr.*, **84** : 223-30, 1954.

229. HORST, K., *et al.* — The effect of habitual use of coffee or decaffeinated coffee upon blood pressure and certain motor reactions of normal young men. *J. Pharmacol.*, **52** : 322-37, 1934.

230. HORST, K., R. J. WILLSON and R. G. SMITH. — The effect of coffee and decaffeinated coffee on oxygen consumption and blood pressure. *J. Pharmacol.*, **58** : 294-304, 1936.

231. SEIBOLD, M. — Die Wirkung des Kaffees auf den Kreislauf aus klinischer Sicht. *Die Heilkunst.*, **2** : 61-65, 1965.

232. SCHWEISHEIMER, W. — Coffee and the heart. *Coffee and Tea Ind.*, **81** : 16-43, 1958.

233. SCHWEISHEIMER, W. — Coffee and low blood pressure. *Coffee and Tea Ind.*, **81** : 48-67, 1958.

234. VALLE, J. R.. and R. F. MELLO. — Effects on metabolism, pulse and arterial pressure. *Brasil Med.*, **53** : 8-10, 1939.

Cardiac function

Cardiac output

235. ATZLER, E. and G. LEHMANN. — Experiments on the effects of caffeine on the healthy heart. *Med. Welt.*, **8** : 525, 1934.

236. DRETTNER, B. — Vascular reactions on the intake of food and drink of various temperatures. *Acta Otlaryng.* (Stockholm), *Suppl.*, **188** : 249-57, 1964.

237. GROBER. — Coffee as a cardiac stimulant for athletics. *Schweiz. Med. Wochenschr.*, **63** : 91, 1933.

238. MATTHIAS, E. — Effect of ordinary coffee and « Kaffee Hag » on function capacity of circulatory organs before and after athletic sports. *Munch. Med. Wchnschr.*, **81** : 555, 1934.

239. NEUTHARD, A. and E. HOEN. — Pharmacological studies on the minute volume of normal human hearts. I. The effect of caffeine, theobromine, theophylline, coffee and Kaffee-Hag. *Arch. Exptl. Path. Pharmakol.*, **185** : 293-301, 1937.

240. POTTHOFF, F. and G. BERENS. — Functional studies in the field of maximum activity. The effect of caffeine on circulation during bodily work. *Z. Ges. Inn. Med.*, **6** : 616-20, 1952.

241. STEPP, W. — Coffee and caffeine as poisons and as drugs. *Arch. Exptl. Path. Pharmakol.*, **190** : 156-70, 1938.

Cardiac arrhythmias

242. BENNETT, I. L. and W. F. WALKER. — Cardiac arrhythmias following the use of large doses of central nervous system stimulants. *Amer. Heart J.*, **44** : 428-31, 1953.

243. GRANATA, G. — Experimental study of the cardiovascular reaction to normal coffee and caffeine-free coffee in children. *Minerva Pediat.*, **5** : 915-22, 1953.

244. MATHIEU, L., S. HADOT and E. HADOT. — Cardiac repercussions of coffee drinking. *Actualities Cardiol. Angeiol. Int.*, **11** : 219-26, 1962.

245. NATHANSON, W. H. — Effect of drugs on cardiac standstill induced by pressure on the carotid sinus. *Arch. Internatl. Med.*, **51** : 387-402, 1933.

246. POLONOVSKI, M., *et al.* — The comparative effects of coffee and soluble extracts of coffee on normal persons and on cardiacs. *Cardiologia*, **21** : 809-16, 1942.

247. REED, C. E., M. F. DRIGGS and C. C. FOOTE. — Acute barbiturate intoxication : a study of 300 cases based on a physiologic system of classification of the severity of intoxication. *Ann. Internal Med.*, **37** : 290-303, 1952.

248. STORZ, H. — Über die Wirkung des Coffeins auf das digitalisierte Herz. *Zeitschr. Ges. Innere Med.*, **4** : 41-50, 1949.

Effects on specific vascular beds

249. AIZAWA, T. and S. HAMAYA. — Cerebral sclerosis and cerebral circulation. *Japan Heart J.*, **2** : 133-46, 1961.

250. DMITRENKO, L. F. — Caffeine (use) in morning headaches in hypertensive disease. *Vrachebnoe Delo*, **27** : 545-50, 1947.

251. DREISBACH, R. and C. PFEIFFER. — Caffeine withdrawal headache. *J. Lab. Clin. Med.*, **28** : 1212-19, 1943.

252. EICHLER, K., *et al.* — Experimental studies on the question of the behavior of sensory and vasoconstrictor stimulus threshold. The effects of coffee and coffee-containing substances on the heart, circulation and blood vessel system. *Arztl. Forsch.*, **18** : 598-603, 1964.

253. FUORTES, M. G. F. and P. E. MASPES. — Therapeutics of intracranial hypertension. *Riv. Patol. Nervosa e Ment.*, **63** : 208-24, 1944.

254. HEUPKE, W. — Effect of drugs on the cerebral vessels in man. *Z. Ges. Exptl. Med.*, **44** : 198-214, 1924.

255. KOUNTZ, W. B. — Studies on the coronary arteries of the human heart. *J. Pharmacol.*, **45** : 65-76, 1932.

256. LIEBERMAN, T. Yu. — Effects on cerebrovascular tonus caused by some headache remedies in hypertensive patients. *Farmikol i. Toksikol.*, **26** : 573-78, 1963.

257. MALOFF, G. — Pharmacological investigations of isolated human veins. *Arch. Ges. Physiol.*, **229** : 38-42, 1931.

258. MODELL, W. — Clinical pharmacology of antianginal drugs. *Clin. Pharm. Therap.*, **3** : 97-108, 1962.

259. RAPHAEL, T. and J. M. STANTON. — Action of certain drugs on brain circulation in man. *Arch. Neurol. Psychiatr.*, **2** : 389-92, 1919.

260. SAVADSKII, S. P. — Experiments on the coronary vessels of the human heart. *Russ. J. Physiol.*, **3** : 219-30, 1921.

261. SCARSELLI, E., *et al.* — The action of coffee and of intravenously and intracarotidally administered caffeine on the cerebral circulation of man. *Giorn. Gerontol.*, **8** : 776-88, 1960.

262. SCHWEISHEIMER, W. — Role of caffeine in relief of headache. *Coffee and Tea Trade J.*, **110** : 24, 1956.

263. SUGIYAMA, S. — On the study of the pressure volume curves of the peripheral blood vessels. *Med. Univ. Imp.* Kioto, **2** : 579-86, 1929.

264. TRUSSEWITSCH, B. I. — Über die Wirkung des Papaverins und einiger anderer gefässerweiternder Gifte auf die Coronargefässe des isolierten Menschenherzens. *Zeitschr. Ges. Exptl. Med.*, **78** : 167-76, 1931.

265. WEPF, R. and RAAFLOUB. — Zur Frage der pharmakologischen Beeinflussung der Gehirngefässe des Menschen. *Helv. Med. Acta*, **17** : 159-66, 1950.

Coffee, caffeine and coronary artery disease

266. BELLET, S., J. ASPE, A. KERSHBAUM and D. ZANUTTINI. — Effect of caffeine on free fatty acids. *Circulation*, **30** : Suppl. 3, III-46, 1964.

267. BELLET, S., A. KERSHBAUM and J. ASPE. — The effect of caffeine on free fatty acids. *Arch. Intern. Med.*, **116** : 750-52, 1965.

268. BELLET, S., A. KERSHBAUM and M. E. FINCK. — Effect of caffeine on free fatty acids. *J. A. M. A.*, **196** : 206, 1966.

269. CLOETONE, W. — Remote therapeutic results of the plant steroid cafesterol in four cases of rheumatismal carditis. *Bruxelles Med.*, **34** : 645-60, 1954.

270. FURST. — Die Gefahren des Kaffees bei Herz- und Arterien-Leiden. *Deutsche Medizinische Presse*, **9** : 91, 1905.

271. GORECKI, Z. — Recherches comparées sur l'action de la caféine, de la strychnine, du cardiazol, de la coramine et de l'huile sur l'appareil circulatoire et respiratoire des cardiaques décompensés. *Bull. Internat. Acol. Polonaise Sce et Leth. Cl. Med.* 317-31, 1930.

272. IZSO, Z. — The effect of caffeine on the blood pressure of cardiac and kidney patients. *Orv. hetil.*, **93** : 752-54, 1952.

273. IZSO, Z. and I. ZARDAY. — The effect of caffeine on blood pressure of persons without vascular disease. *Orv. hetil.*, **93** : 755-56, 1952.

274. LEE, S. — Coffee related to coronary disease? *Tea and Coffee Trade J.*, **128** : 18, 74, 76-78, 80, 82-83, 1964.

275. LEVY, R. — Coffee as a cause of cardiac pain. *Ann. Internal Med.*, **11** : 833-37, 1937.

276. MYASNIKOV, A. L. — The influence of some neurotropic drugs on the cholesteremia of atherosclerotic patients. *Klin. Med.*, **34** : 65-69, 1956.

277. PAUL, O., *et al.* — A longitudinal study of coronary heart disease. *Circulation*, **28** : 20-31, 1963.

278. SHARLAI, R. I. — Physiological basis for use of bromide, caffeine and calcium chloride in cardiovascular pathology *Vrachebnoe Delo*, **10** : 1009-14, 1957.

279. TURNER, C. — According to Camp Hill physician-heavy coffee drinkers « living dangerously ». *Patriot*, Thursday, Oct. 14, 1965 (newspaper, Harrisburg, Pa.) no page number.

280. YUDKIN, J. — Dietary fat and dietary sugar in relation to ischemic heart disease and diabetes. *Lancet*, **2** : 4-5, 1964.

281. YUDKIN, J. and J. RODDY. — Levels of dietary sucrose in patients with occlusive atherosclerotic disease. *Lancet*, **2** : 6-8, 1964.

282. SCHROEDER, H. A. — Cadmium, chromium, and cardiovascular disease. *Circulation*, **35** : 570-580, 1967.

283. SCHROEDER, H. A., A. P. NASON, I. H. TIPTON, and J. J. BALASSA. — Essential trace metals in man : zinc. Relation to environmental cadmium. *J. chron. Dis.*, **20** : 179-210, 1967.

RIKER (W.), KENSLER (C. J.). — **Les effets cardiovasculaires de la caféine et du café chez les animaux et l'Homme.** Troisième Colloque International sur la Chimie des Cafés Verts, Torréflés et leurs Dérivés, Trieste, 2-9 juin 1967. A.S.I.C. (Paris) 1968, p. 351-373, 283 réf.

La caféine et le café ont une action marquée sur le système cardiovasculaire et agissent suivant des principes pharmacologiques connus :

— la réaction varie d'un individu à l'autre dans une population normale ;

— l'effet est proportionnel à la dose, mais pas de façon frappante : la différence entre l'effet d'une et deux tasses ou de deux et trois tasses de café peut ne pas être immédiatement détectée lors d'expériences sur le système circulatoire de la majorité des individus normaux ;

— certaines drogues créent un état d'accoutumance tel que l'effet initial diminue ou disparaît à la suite d'absorptions répétées. C'est le cas de la caféine,

RIKER (W.), KENSLER (C. J.). — **Cardiovascular actions of caffeine and coffee in animals and man.** Troisième Colloque International sur la Chimie des Cafés Verts Torréflés et leurs Dérivés, Trieste 2-9 juin 1967. A.S.I.C. (Paris) 1968, p. 351-373, 283 réf.

It is clear that caffeine and coffee interact with the cardiovascular system in accordance with well-known pharmacologic principles. Foremost among these is the variability of response among individuals from a normal population. A second principle, that the effect is proportional to dose, is also evident, though not strikingly so. The practical meaning is that the difference between one and two, or two and three, cups of coffee might not be readily detected in tests on the circulatory systems of most normal men.

A third pharmacologic principle is that some drugs induce a state of tolerance, in which the original effect diminishes or disappears upon repeated usage. This is so for caffeine. But we do not know how long it takes to acquire

RIKER (W.), KENSLER (C. J.). — **Die kardiovaskuläre Wirkung des Coffeins und des Kaffees beim Tier und beim Menschen.** Troisième Colloque International sur la Chimie des Cafés Verts, Torréflés et leurs Dérivés, Trieste, 2-9 juin 1967. A. S. I. C. (Paris), 1968, p. 351-373, 283 réf.

Coffein und Kaffee haben eine ausgeprägte Wirkung auf das kardiovaskuläre System und wirken gemäss bekannten pharmakologischen Grundsätzen :

— die Reaktion ist von einem Menschen zum anderen bei einer normalen Bevölkerung verschieden ;

— die Wirkung ist proportional mit der Dosis, jedoch nicht auf auffällige Weise : der Unterschied zwischen der Wirkung von einer und zwei oder von zwei und drei Tassen Kaffee kann bei den meisten normalen Menschen nicht immer sofort bei Experimenten am Kreislaufsystem wahrgenommen werden ;

— gewisse Drogen bewirken einen derartigen Toleranzzustand, dass die ursprüngliche Wirkung bei wiederholter Einnahme abnimmt oder ganz verschwindet. Dies ist der Fall bei dem

mais on ne sait pas combien de temps il faut pour acquérir cette tolérance et combien de temps il faut pour la perdre.

En résumé, les réactions cardiovasculaires à la caféine ou au café sont négligeables chez la majorité des gens ; seuls les individus peu tolérants présentent des réactions marquées. On ne sait virtuellement rien au sujet des effets cardiovasculaires chroniques. Il apparaît que l'abus répété du café provoque une arythmie cardiaque chez une petite fraction de la population. Les effets hystopathologiques de l'administration chronique de caféine sont incertains chez l'animal et inconnus chez l'Homme, tout au moins en ce qui concerne le système cardiovasculaire. Bien que le niveau de la consommation de café puisse être un des facteurs liés à l'ischémie du cœur, l'interaction de nombreux facteurs reste à déterminer.

Si les effets cardiovasculaires marqués du café semblent largement dépendre de la teneur en caféine, on ne peut pas dire que les autres éléments soient inactifs ; ils n'ont pas été étudiés. Il semble donc très souhaitable d'étudier les effets marqués et chroniques des composants du café, autres que la caféine.

caffeine tolerance, or, once acquired, how long it takes to lose it.

In sum, all the preceding principles probably underlie the fact that cardiovascular reactions to caffeine/coffee are negligible in the majority of the normal population.

Intolerant individuals generally show more marked cardiovascular reactions to caffeine-containing beverages.

Virtually nothing is known about chronic cardiovascular effects. Functionally, it appears that chronic abuse of coffee will provoke cardiac arrhythmias in a small fraction of the human population. Histopathological effects of chronic caffeine administration are uncertain in animals, and unknown in man, at least with respect to the cardiovascular system. Though the degree of coffee consumption may be one of many associative factors in some patients with ischemic heart disease, the interaction of the multiple factors is obscure.

Whereas the acute cardiovascular effects of coffee appear to depend largely on the caffeine content, we cannot say that the other components are inert. They have simply not been investigated. Therefore it seems highly desirable to establish both the acute and chronic effects of coffee components other than caffeine.

Coffein. Es ist jedoch nicht bekannt wieviel Zeit es braucht, um diese Toleranz zu erreichen und wieviel Zeit um sie zu verlieren.

Zusammenfassend kann gesagt werden, dass die kardiovaskuläre Reaktionen auf den Coffein oder den Kaffee bei den meisten Menschen belanglos sind ; nur Menschen die eine geringe Toleranz aufweisen zeigen eine ausgeprägte Reaktion. Man weiss virtuell nichts über die chronische kardiovaskuläre Wirkung. Wiederholter übermässiger Genuss von Kaffee scheint bei einem kleinen Teil der Bevölkerung zu Herzarythmie zu führen. Die histopathologische Wirkung der chronischen Verabreichung von Coffein ist beim Tier ungewiss und beim Menschen unbekannt, wenigstens was das kardiovaskuläre System anbelangt. Obwohl die Menge eingenommenen Kaffees ein mit Herzischämie verbundener Faktor sein kann bleibt die Wechselwirkung zahlreicher Faktoren noch festzusetzen.

Wenn auch die ausgeprägte kardiovaskuläre Wirkung des Kaffees weitgehend vom Coffeingehalt abzuhängen scheint, kann nicht gesagt werden, dass die übrigen Grundstoffe unwirksam sind, nur wurden sie nicht untersucht. Es empfiehlt sich daher, die ausgeprägte und chronische Wirkung der übrigen Komponenten des Kaffees ausser dem Coffein ebenfalls zu untersuchen.

DISCUSSION

M. NAVELLIER : Je voudrais simplement attirer l'attention sur un détail qu'a signalé le Dr Kensler, détail qui me paraît très important : au cours des études qui ont été rapportées, on a pris en considération le nombre de tasses de café consommées par les sujets en cause. Le Dr Kensler a remarqué qu'il semblait y avoir une différence dans l'action du café selon que celui-ci était plus ou moins torréfié. Je pense qu'il entre tout à fait dans les attributions de notre association d'étudier d'une part comment les différents composants du café peuvent avoir un effet physiologique, et d'autre part comment tous les facteurs qui influent sur cette composition du café, depuis l'origine de la plante jusqu'à la technologie et à la préparation de la boisson, sont également en fin de compte des facteurs qui influent sur les effets physiologiques. Nous avons à notre programme un rapport de M. Adrian sur les différentes teneurs en vitamine PP selon l'origine du café et sa préparation. Je pense que ce genre d'études devrait être en corrélation étroite avec les études physiologiques et à ce point de vue, entre autres, le rapport du Dr Kensler me semble d'un intérêt extrêmement élevé.

M. LANG : Sie haben betont, dass es wünschenswert ist, dass auch die Begleitstoffe des Coffeins im Kaffee hinsichtlich ihrer akuten und chronischen Wirkung auf das Herz-Kreislaufsystem untersucht werden und haben auch schon die mögliche Bedeutung von Zucker und Cadmium erwähnt. Darf ich fragen, ob Sie schon einen bestimmten Verdacht auf die Beteiligung anderer Substanzen oder Substanzgruppen haben ?

M. KENSLER : After listening to the analytical chemists finding so many new compounds, I suppose that some of them may be biologically active at certain doses. But the one thing that I noticed is that nobody reported how much was present.

However, I think that in that field of circulation, there are materials such as chlorogenic acid affecting rates of absorption and peak concentration levels which may be important.

M. ADRIAN : Je voudrais savoir si l'on dispose de statistiques concernant les effets physiologiques et cardiovasculaires du café en fonction des niveaux alimentaires des populations, notamment dans les régions où la ration est riche en matières d'origine animale et celles où la ration est essentiellement végétale ?

M. KENSLER : With respect to the question about variation of the activity of caffeine dependant on the nutritional status of the population, we have not found any publication which throws any light on this at all. Perhaps some of the experiments on rats could give an idea.

M. PAOLETTI : At least in the rats, the effects of the methylxanthines are much more pronounced in starving rats than in well fed rats.

M. CZOK : Ich möchte noch auf den folgenden Befund hinweisen : Prof. Heyden hat bei Kaninchen durch Verabreichung einer entsprechenden Diät eine Erhöhung des Cholesterinspiegels im Blut gefunden und konnte ausserdem nachweisen, dass durch Verabreichung von Kaffee eine deutliche Senkung des erhöhten Cholesterinspiegels hervorgerufen wird.

M. KENSLER : All that I am familiar with is an experiment on rabbits involving cholesterol levels.

When 4-5 % cholesterol was administered in the diet, plaques of cholesterol appeared in the vessels. The administration of caffeine decreased the incidence. So one might think that caffeine should tend to reduce the coronary disease. But unfortunately we have not that kind of epidemiologic data.

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MUTAGENICITY STUDIES IN MICE AFTER CHRONIC TREATMENT WITH CAFFEINE*)

By G. RÖHRBORN

With 10 Tables.

Caffeine belongs to those substances of the human environment which are consumed by a large part of the population during a prolonged period of time. Information on the mutagenetic effect of such substances in particular is extremely important. Accordingly, numerous mutagenicity investigations with caffeine have been performed. Thus MAINX reported as early as 1929 on a caffeine-induced mito-visual obstruction with Tradescantia pillosa. KIHLMAN and LEVAN verified a similar effect in 1949 with Allium cepa. They additionally found a caffeine-induced inhibition of the cell wall formation and chromosome aberrations. With Escherichia coli DEMEREC et al. (1951), GEZELIUS and FRIES (1952), GREER (1958), NOVICK (1956), KIBUTSCHECK and BENDIGKEIT (1964) observed the mutagenic effect of caffeine. FRIES and KIHLMAN (1948) were able to determine the mutagenic effect of caffeine in fungi. Results with Drosophila are contradictory. Accordingly, ANDREW (1959) found a slight mutagenic activity, but could not detect any specifically sensitive stages of the spermatogenetic cycle. A few years later YANDERS and SETON found no mutagenic caffeine effect with Drosophila, under similar test conditions. The studies of ALDERSON and KHAN (1967) with Drosophila also yielded negative results. On the other hand, OSTERTAG and HAAKE (1966) verified mutagenic effects after administering caffeine to Drosophila larvae.

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MITTLER and collaborators (1967) furthermore reported an increase of the nondisjunction-rate after caffeine administration to Drosophila. The above is in turn contradicted by the findings of CLARK and CLARK (1968) who verified a reduction of the nondisjunction frequency and who failed to detect any additional indication for the induction of X-chromosomal recessive lethal mutations in Drosophila. On the other hand, these authors found indications of increased chromosome loss in the caffeine-series.

In HeLa-cell cultures and lymphocyte cultures, caffeine-induced chromosome disjunctions were found by OSTERTAG and collaborators, but only a few reunion forms, i.e. chromosome mutations in a stricter sense of the term were verified by them.

Consideration of mammal metabolism, "cum grano salis", is especially significant when evaluating the possible mutagenic effect of a substance. Acute therapy with high caffeine doses in mice did not reveal a mutagenic effect, either in cytogenetic investigation, or when examining spermatocytes from the viewpoint of chromosome aberrations (ADLER, 1966). Since man usually consumes caffeine chronically, tests were likewise performed to determine the mutagenic effect with the aid of chronic caffeine-therapy. The findings, however, are not conclusive and mostly negative (BLUHM,1924; CATTANACH,1962, 1964; EICHLER et al., 1932; KUHLMANN, 1967; KUHLMANN at al., 1962; STIEVE, 1928).

The principal purpose of present investigation was an extensive mutagenicity study after long-term therapy with drinking water containing caffeine in low dosage ranges , since it is highly probable that threshold value effects in the chemical mutagenesis play a significant role.

Material and Method

We treated male mice of the C3H inbred strain and (101xC3H)-F_1-hybrids with three different caffeine-concentrations in drinking water. The therapy was started at the age of 3 weeks. All animals originated from our own breed and were kept in completely climate-controlled animal quarters at 22°C and an air humidity of 50 to 55%. The parallely tested controls received only tap water for drinking.

A substantially smaller group of C57BL mice, made available at the age of 6 weeks by Dr. KUHLMANN, received drinking water of high caffeine concentration. We additionally administered an identically high caffeine concentration as used for the C57BL animals, to a group of our own C3H inbred-♂♂. The quantity of the drinking water intake and the body weight of the animals was determined during a treatment period of 245 and 246 days respectively, or in a single case even during 351 days. The synopsis of the verified values is shown on Table 1. Thereby the two low physiologic concentrations were administered up to a total continued period of 550 days to the C3H- and (101xC3H)F_1-♂♂.

In each case, 3♂♂ stayed in a cage of the type II (Altronin). As Table 1 shows, the mice of the strains C3H and (101xC3H)F_1, treated with the 0,0023 caffeine solution, consumed approximately 3,4 to 3,6 mg caffeine per kg of body weight daily. This corresponds approximately to the caffeine content og 2 to 3 cups of coffee. In the next-highest caffeine series, (0,01%) the C3H ♂♂ consumed an average of 14,9, and those of the (101xC3H)F_1♂♂ consumed an average of 17,3 mg caffeine per kg body weight. That corresponds to more than 10 cups of coffee per day. According to the guidelines of the Association of Food and Drug Officials in the United States (1959), the above dose in the mouse is still comparable to the daily dose consumed by man.

No comparison is possible, however, with the 0,5% caffeine concentration. Said overdose was intentionally selected for a safer evaluation of test results in case of a negative test outcome. No change in drinking behavior was observed as a consequence of caffeine content in the drinking water in the two physiologic concentrations, as evindet from Table 1. The drinking water intake was reduced by the caffeine content only in case of the highest, "non-physiologic" concentration of caffeine. A correction of the drinking water intake during the overdosage-test in question was intentionally dispensed with, so as to permit the simultaneous determination of toxic side-effects which could promote the release of dominant lethal mutations through caffeine.

At specific periods of time after the treatment, the treated and untreated ♂♂ were mated with 10 to 12 weeks-old virgin ♀♀ of the same strain in each case. Approximately on the 15th day of pregnancy they were dissected, the corpora lutea graviditatis were counted and the number of the living and dead implants was counted after opening of the uterus. According to our earlier specifications (ROHRBORN, 1968, 1970 a), we considered in particular an increase of dead implants following the treatment of male animals as a criterion for induced dominant lethal mutations. Pre-implantation ovum loss alone, however, is not believed by us to represent an unequivocal and sufficient criterion for dominant lethal mutations, since such a loss could also be attributed, among others, to high-grade oligosperms or to the inactivation of sperm motion.

The χ^2-method was applied for the statistical calculation of the test results.

Nutrition Science Journal, Supplement 14.

TABLE 1.

Average daily intake of various caffeine concentrations in the drinking water of male mice.

1 Stamm	2 % Coffein-Konzentration	n ♂♂	3 Coffein-Aufnahme in Tagen	4 durchschnittliche Aufnahme cc/g Körpergewicht pro Tag	5 mg Coffein / kg Körpergewicht pro Tag
(101 × C3H)F_1	0,0023	96	245	0,1475	3,39
(101 × C3H)F_1	Kontrolle 6	39	245	0,1359	–
(101 × C3H)F_1	0,01	18	246	0,1723	17,23
(101 × C3H)F_1	Kontrolle	6	246	0,1225	–
C3H	0,0023	90	245	0,1555	3,58
C3H	Kontrolle	24	245	0,1555	–
C3H	0,01	81	246	0,1492	14,92
C3H	Kontrolle	18	246	0,1292	–
C3H	0,5	15	351	0,0010	304,92
C3H	Kontrolle	6	351	0,0907	–
C57BL	0,5	7	245	0,1029	514,33
C57BL	Kontrolle	4	245	0,1261	–

1) Strain. 2) Caffeine concentration %. 3) Caffeine intake in days.

4) Average intake per day, cc/g of body weight.

5) $\frac{\text{mg caffeine}}{\text{kg body weight per day.}}$ 6) control

Results.

1. Treamanet of C57Bl-♂♂

In the course of a minor orientation test regarding the sensitivity of C57BL-mice we treated 11 C57BL-♂♂ with the extremely high 0,5% caffeine concentration in drinking water. For control purposes 4 C57BL-♂♂ received simultaneously tap water. As mentioned earlier, therapy in this case began at the age of 6 weeks after receipt of the animals from Dr. KUHLMANN. 101 days later we interrupted the therapy for the duration of 8 weeks and mated each male with 3 virgin female mice (101×C3H)F_1, 10 to 12 years old. The results of the dominant lethal test connected with the above are shown on Table 2.

As evident from Table 2, after 161 days the pre-implantation ovum losses in the caffeine-series were higher as compared with the controls. The difference was statistically significant ($P \sim 0,027$). On the other hand, the rates of dead implants (relatively to the total number of implants) in the caffeine-series was even slightly lower than the rate for the simultaneously checked controls.

After the above 6-week cross-breeding test, the treatment of the same animals was continued up to a total duration of 245 days, using the same caffeine concentration. The same males were once more used as controls. This was followed once more by cross-breeding with $(101xC3H)F_1$ females, aged 10 to 12 weeks. Table 2, bottom line, shows the results. It was found that neither the pre-implantation ovum losses nor the quotas for dead implants in the caffeine series exceeded the control values.

2. Treatment of C3H-♂♂

Table 1 shows that we treated 90 C3H-♂♂ with a 0,0023% caffeine concentration, 81 C3H-♂♂ with a 0,01% caffeine concentration and 14 C3H-♂♂ with a 0,5% caffeine concentration. After specified intervals they were cross-bred with 10-12 weeks old, untreated virgin ♀♀ of the same strain. Therapy was discontinued during this period. The mating duration in the two "physiologic" caffeine ranges was 2 weeks only, since a large number of ♂♂ was available. The 0,5% caffeine-series was mated (spliced) for the duration of 8 weeks, so as to increase the material, since only a small number of treated ♂♂ were available in this series.

Table 3 includes a synopsis of the single values, after it was found during a heterogeneity test that the results for the single weeks did not differ significantly from each other ($P > 0,03$ according to BRAND-SNEDECOR). As shown by Table 3, the only conspicuous difference between test and control is found in the 0,01% caffeine series after 91 days of therapy. The P-value for differences between dead implant quotas amounts to 0,015.

TABLE 2. Test results with the dominant lethal mutation method after chronic treatment of C57BL-♂♂[1] with extremely high caffeine concentrations in the drinking water.

1 Trinkwasser	2 Behandl. in Tagen	n 2/A befr. ♀♀	n Corp. lut.	n Impl.	n Impl. pro ♀	n leb. 3 Embr.	n leb. 4 Embr. pro ♀	% PE[2] probability	n TI[3]	% TI[4] probability
0,5% Coffein	161	62	607	465	7,50	428	6,90	23,4 } P ~ 0,0027	37	8,0 } P ~ 0,8
Kontrolle	161	15	148	130	8,67	119	7,93	12,2	11	8,5
0,5% Coffein	245	48	444	375	7,81	350	7,29	15,5 } P ~ 0,26	25	6,7 } P ~ 0,74
Kontrolle	245	10	99	88	8,80	83	8,30	11,1	5	5,7

1) Drinking water. 2) Treatment in days. 2/A) n(number) of fertilized ♀♀. 3) Living embryos, number of, per ♀. 5) 0,5% caffeine controls

1) mated with 10-12 weeks old (101 x C3H)F_1-♀♀.
2) PE=pre-implantation ovum loss.
3) TI = dead implants
4) in % of the total implant number.

As stated already in 1968 (RÖHRBORN,1968), however,during investigations aimed at the release of dominant lethal mutations only those P-values should be evaluated as criteria for significant differences between test and control which are below 0,0027. It was therefore suspected from the start that said difference is of an accidental nature. The above assumption is reinforced by the fact that the P-value increases after the 245-day treatment (~0,20), which could admittedly be connected with the small number of the implants. Neither the test with the lowest concentration (0,0023%) nor the test with the highest concentration (0,5%) yields additional definite indications for the induction of dominant lethal mutations. On the contrary, the P-values for the dead implants, listed on Table 3, are instead rather indicative of test and control homogeneity.

For the purpose of problem clarification we continued treatment with the two low caffeine concentrations up to a total duration of 550 days. After said period the treated ♂♂ and their controls were in each case again mated with 10 to 12 weeks old virgin ♀♀ of the same strain, and the quotas of pre-implantation ovum losses and of dead implants were determined.

TABLE 3. Test results with the dominant lethal mutation method after chronic treatment of C3H-♂♂ with 3 caffeine doses.

1 Versuch	2 Behandl.-dauer	3 n c.l.	n Impl.	% PE[1])	n TI[2])	% TI[3])	probability	% PE + TI[4])	probability
0,0023% Coffein	245	877	802	8,6	99	12,3	~ 0,36	19,8	~ 0,66
Kontrolle	245	242	219	9,5	22	10,0		18,6	
0,01% Coffein	91	2163	1911	11,7	268	14,0	~ 0,015	24,0	~ 0,03
Kontrolle	91	649	577	11,1	58	10,1		20,0	
0,01% Coffein	245	679	603	11,2	76	12,6	~ 0,20	22,4	~ 0,07
Kontrolle	244	130	120	7,7	10	8,3		15,4	
0,5% Coffein	146	820	741	9,6	136	18,4	~ 0,68	26,2	~ 0,44
Kontrolle	146	335	307	8,4	53	17,3		24,2	
0,5% Coffein	351	1460	1292	11,5	177	13,7	~ 0,90	22,1	~ 0,2
Kontrolle	351	796	727	8,7	101	13,9		21,4	
						χ^2 = 8,92		χ^2 = 9,93	
						P [5] ~ 0,10		P [5] ~ 0,08	

1) Test. 2) Duration of treatment. 3) number (n)
4) . . caffeine
control

1) Pre-implantation ovum loss in % of the corpora lutea counts
2) TI = dead implants
3) in % of the total implant number
4) in % of the corpora lutea count.

TABLE 4. Pre- and post-implantation ovum losses after 550 days of coffein treatment administered to C3H -♂♂.

	n 1) bofr. ♀♀	n c.l.	n Impl.	% PE	probability	% TI	probability
0,0023% Coffein	87	907	755	16,9	> 0,3	11,0	< 0,6
Kontrolle	19	188	163	13,3		12,3	
0,01% Coffein	46	419	355	12,9	> 0,8	13,8	> 0,2
Kontrolle	8	75	63	10,7		19,0	

1) Number of fertilized ♀♀

As Table 4 shows, in two of the caffeine-series the pre-implantation ovum losses exceeded the controls slightly; on the other hand, the quotas for dead implants in the caffeine-series were slightly below those for the controls; the differences are not significant in any sense. The homogeneity between test- and control-series becomes even more definite when the number of living embryos per fertilized ♀ is subjected to comparison (Table 5).

TABLE 5.

Test	number living embyos per fertilized C3H-♀
0,0023 % caffeine	
550 days	7,72
control	7,62
0,01 % caffeine	
550 days	6,65
control	6,38

Since the average number of living embyos per fertile ♀ in both caffeine-series is even somewhat higher than the number of the parallel controls, this argues against the induction of dominent lethal mutations by means of the treatment.

3. Treatment of (101xC3H)F_1-♂♂.

The treated animals of the 0,01% caffeine-series and their controls were in each case cross-bred after 105 days for 1 day, and after 246 days for 8 weeks with 3 virgin ♀♀ in each case, aged 10 to 12 weeks, of the same type. The results of each week were in turn summarized. The animals of the 0,0023% caffeine series were mated to virgin ♀♀ aged 10 to 12 weeks, of the same strain, after 250 days of treatment, for the duration of 2 weeks. Since the results for single weeks proved again to be heterogeneous, the single test series and their controls are synptically represented on Table 6.

TABLE 6. Test results with the dominant lethal mutation method after chronic treatment of (101XC3H)F_1- ♂♂[1] with low caffeine doses.

1 Versuch	2 Behandlungsdauer in Tagen	3 n befr. ♀♀	n c.l.	n Impl.	4 n Impl. pro befr. ♀	5 n leb. Embr.	6 n leb. Embr. pro ♀
0,0023% Coffein	245	171	1794	1569	9,18	1461	8,54
Kontrolle	245	71	716	645	9,08	590	8,44
0,01% Coffein	105	39	410	364	9,33	336	8,62
Kontrolle	105	13	125	116	8,92	106	8,15
0,01% Coffein	246	257	2509	2268	8,90	2113	8,22
Kontrolle	246	93	890	804	8,65	748	8,04

1) Test. 2) Duration of treatment in days. 3) Number of fertilized ♀♀. 4) number of implants per fertilized ♀. 5) Number of living embyos, per ♀.

1) At the start of the treatment all ♂♂ were 3 weeks old. After the treatment the ♂♂ were mated with 10 to 12 weeks old (101 X C3H)F_1- ♀♀.

Table 6 definitely shows the following two facts:

1. The number of the implants per fertilized ♀ is even higher for each caffeine-series than for the corresponding control-series. This implies that no caffeine-induced, pre-implantation ovum loss has taken place.

2. The number of living embryos per fertilized ♀ is likewise slightly higher for each caffeine-series than for the corresponding control-series. This makes it evident that no caffeine-induced implant death occurred here either as a result of the treatment.

Two different types of errors can occur in the course of the mutation experiment. The first error consists in the definition of a compound as mutagenic when no sufficient confirmation is given ($P > 0,0027$ for dominant lethal experiments). The second possible error consists in the definition of a compound as non-mutagenic when weak mutagenicity is in fact present. Apart from the lack of significance for induced dominant lethal mutations, we therefore asked ourselves how reliable the negative finding

of these tests, seemingly so convincing, actually is. We were able to demonstrate according to the test procedure of STROBEL and VOGEL (1958) that merely tests with the highest dose already exclude a caffeine-induced increase of the pre- and post-implantation ovum loss rates in excess of the factor 1,16 (95% confidence limit).- We neverthless continued to treat these ♂♂ as well, up to a total duration of 550 days. We then mated the animals of the 0,0023% series for a period of 3 weeks, and those of the 0,01% series for 8 weeks (for the purpose of material increase), once more with 10 to 12 weeks old virgin ♀♀ of the same type; the single series were summarized after a heterogeneity test (Table 7).

TABLE 7. Pre- and post-implantation ovum losses after 550 days of caffeine treatment administered to $(101xC3H)F_1$- ♂♂.

	1 n befr. ♀♀	n c.l.	n Impl.	% PE	probability	% TI	probability
0,0023% Coffein	135	1323	1190	10,1	> 0,2	7,7	> 0,8
Kontrolle	50	498	437	12,2		8,0	
0,01% Coffein	29	274	245	10,6	~ 0,7	11,4	0,05-0,1
Kontrolle	8	77	70	9,1		4,3	

1) Number of fertilized ♀♀.

As Table 7 demonstrates, no definite indications are in evidence for induced dominant mutations. This suggestion becomes in turn more definite when comparing average numbers of living embryos per fertilized ♀ listed on Table 8.

TABLE 8

Test	Number living embryos per (101xC3H)F_1-♀
0,0023 5 caffeine 550 days	8,1
control	8,0
0,01 % caffeine 550 days	7,5
control	8,4

4. Treatment of C3H- and (101XC3H)F_1-♀♀

Since it is possible that female individuals could be more sensitive than males to the induction of dominant lethal mutations through caffeine, we treated female mice for a period of 43 weeks with drinking water containing 0,01% caffeine. The parallely tested controls received standard tap water. The treatment began at the age of 3 to 4 weeks. After discontinuation of the therapy the animals were mated with untreated, 10 to 12 weeks old ♂♂. The results are displayed on Table 9. It is obvious according to said Table that in both strains, the rates of pre-implantation ovum losses and of the total implants in the controls as well as in the tests were considerably higher than values listed on the other Tables in tests and in controls. The only explanation of said effect is: the high rates for pre-implantation ovum losses and total implants depend on the age of the dam. These findings demonstrate with specific clarity how important homogeneous age groups are in tests and controls during experiments of the type at issue.

TABLE 9. Pre-implantation ovum loss and total of implants after chronic treatment of female mice with a 0,01% concentration of caffeine in the drinking water during 43 weeks.
(Age of the animals at the start of the treatment: 3 to 4 weeks)

TABLE 9 continued:

1 Stamm	2 Paarungszeit in Wochen nach Behandlung	n c.l.		n Impl.		% PE		% TI	
		V	K	V	K	V	K	V	K
C3H	1.	122	58	70	32	42,6	44,8	32,9	34,4
(101 × C3H)F_1	1.	480	243	321	152	33,1	37,4	48,3	44,7
(101 × C3H)F_1	2.	427	120	261	88	38,9	26,7	57,1	70,5

1) Strain. 2) Mating period in weeks, after treatment.

V = test (0,01% caffeine, for 43 weeks)
K = control.

In the course of a minor orientation test we furthermore investigated the question whether acute therapy with a high caffeine dose results in a caffeine-induced increase of the pre-implantation ovum losses and/or of the dead implants. The results are shows on Table 10.

TABLE 10. Preliminary test results with the dominant lethal mutation method, after a single intraperitoneal injection of 0,25 g/kg caffeine, in (101 x C3H)F_1-♂♂.

1 Paarungstage nach Injektion	n 2 befr. ♀♀		n c.l.		n Impl.		% PE		n 3 toto Impl.		% 3 toto Impl.	
	V[3])	K[3])	V	K	V	K	V	K	V	K	V	K
0- 1	8	5	80	55	74	49	7,5	10,9	7	3	9,5	6,1
2- 8	21	9	197	85	173	80	12,2	5,9	18	4	10,4	5,0
9-15	33	10	302	97	274	89	9,3	8,2	27	12	9,9	13,5
16-22	36	10	335	107	294	100	12,2	6,5	26	7	8,8	7,0
23-29	39	9	391	90	351	79	10,2	12,2	37	5	10,5	6,3
30-36	14	9	142	96	124	86	12,7	10,4	6	13	4,8	15,1
Σ 0-36	151	52	1447	530	1290	483	10,9	8,9	121	44	9,4	9,1

1) Mating days after injection. 2) Number of fertilized
3) dead implants, (number . . . %. .)

1) Age during treatment: 10 weeks + 2 days; age of the (101 x C3H)F_1 -♂♂ at the time of the mating: 10 to 11 weeks.
2) V-caffeine series; K = control.

Since tests with Endoxan verified high rates of induced dead implants (ROHRBORN, 1970b), in particular among the ♀♀, fertilized on the first day after discontinuation of the treatment, said day was subjected to specific analysis (first line). For additional analyses, the values were summarized per week. No significant differences became evident either for single weeks or for totals between test and control concerning the rates of dead implants. The rates for pre-implantation ovum losses in the caffeine-series were likewise only insignificantly higher than those of the control. This implies that we were unable to obtain definite indications in favor of the induction of dominant lethal mutations with the acute treatment of female mice.

Discussion

Dominant lethal mutations can become manifest in pre-implantation ovum losses as well as in dead implants. After treating male animals, however, pre-implantation ovum losses can be triggered also by oligospermiae and other non-mutative processes. Therfore "u.E" dead implants are a substantially better criterion for dominant lethal mutations. For the above reason alone those pre-implantation ovum losses which occur after a 161-day treatment of C57BL-♂♂ with 0,05% caffeine solution in drinking water cannot be regarded conclusive for the induction of dominant lethal mutations--since the rates of dead implants failed to show significant differences between test and control. In earlier tests with trypaflavine and hexamethylentetramine (BALDERMANN at al., 1967) it became evident that the weak mutagenicity of a compound manifests itself in the dead implant rates only. This is explainable as follows: the single lethal impacts per germ cell cause in a relatively late embryonal mortality, i.e. dead implants. Strong mutagenics, on the other hand, cause, besides increased dead implant quotas, considerable pre-implantation ovum losses as well. This could be explained by the fact that in the last-mentioned cases several lethal impacts per gamete occurred.

*) "u.E. - unseres Erachtens" - in our opinion

In other words: the more lethal impacts per germ cell occur, the earlier does the embryo atrophy. It seems therefore unlikely that caffeine could cause directly opposite effects, i.e. it is unlikely that caffeine-induced dominant lethal mutations could become effective exclusively during the pre-implantation phase. Furthermore, it should be recalled that after continued treatment, the pre-implantation ovum loss rate difference between test and control was no longer significant.

In tests with C3H- and (101xC3H)F_1- ♂♂ we likewise failed to detect any significant induction of dominant lethal mutations through caffeine. In particular, the tests with hybrid mice (Table 6) exclude induction of dominant lethal mutations by means of caffeine with a satisfactory degree of reliability (see specifications for Table 6). In addition to the above, results obtained after 550-day treatment likewise argue against a caffeine-induced dominant lethality within these two "physiologic"concentration ranges. Our test results coincide well with the caffeine investigations of LYON at al., (1962) and ADLER (1969). No additional definite mutagenicity indications were provided either by the cytogenetic study of ADLER (1966) after acute treatment, or of ADLER and ROHRBORN (1969) after chronic treatment. The material of KUHLMANN at al. (1968) does not seem sufficiently extensive regarding the total number of the implants and the number of dead implants in the single concentration ranges, and specifically regarding the control (52 implants with 8 ♀♀); it can therefore not be conclusive for the induction of dominant lethal mutations by means of caffeine. ADLER (1970) who discusses said study in detail, furthermore expresses a correct criticism when stating that the control was not parallel with the single tests. ADLER mentions additionally that KUHLMANN's test series include those ♀ as well, which had only dead implants. In our view, this could originate rather from an infect of the dam than from the induction of dominant lethal mutations in the sperm cells of the sire.

Furthermore, our negative results coincide well with the findings of VOGEL et al. (1966) who found no indications of a mutagenetic effect from caffeine in the course of an examination in man. Therefore our investigations and those of VOGEL et al. hopefully indicate that--at least in low concentrations--caffeine has no, or only a slight mutagenic effect in mammals and in man. Various questions neverthless remain unanswered:

1. Are the strains used by us especially sensitive to induced caffeine-mutagenesis? This argument is somewhat weakened by the fact that CATTANACH (1962,1964), LYON at al.(1962) and EPSTEIN (1970) likewise failed to find significant indications in favor of caffeine-induced mutations during experiments with other mouse strains.

2. Are mice especially immune to caffeine-mutagenesis? This problem should be clarified by using other species of mammals.

3. What is the situation concerning synergistic effects? WITKIN (1958), LIEB (1961) and LUMB at al. (1968) found, for example, "dark-repair" inhibition of ultraviolet-induced mutations in bacteria by caffeine. HARM (1967) obtained similar results concerning the survival of E. coli after UV-irradiation in the presence of caffeine, and GRIGG (1968) developed a model of caffeine-induced E. coli death by means of chromosome dissociations which occur during the inhibition of repair-enzymes. WRAGG at al. (1967) furthermore observed the inhibition of DNS-polymerase-activity by caffeine in a "Säuger" (mammal?)-cell culture, with concentrations over 200 µg/ml. ADLER and SCHONEICH (1967) reported on the increased incidence of chromosome dissociations in S_2-sarcomas in mice after intraperitoneal injection of pure oxygen and high caffeine doses. Caffeine and oxygen, when administered alone in each case, showed no effect.

On the other hand, CLARK (1968) foun in a schizosaccharomyces test that "caffeine is without any obvious effect on the lethality of met^+ reversions induced by nitrous acid or nitromethylurethane treatments. This suggests that the caffeine-sensitive dark repair system is only specific for some types of lesion". Using the X-ray splitting technique in a case of vicia faba, WOLFF (1968) found no indication that caffeine inhibits the X-ray-induced chromosome dissociations of the repair mechanism. CLEAVER (personal information) likewise failed to detect any inhibition of the repair processes in mammal cell cultures. Finally, EPSTEIN (1970) was unable to determine in mice any synergistic effects of caffeine, methylmethane-sulfonate or X-rays respectively, in the course of dominant lethal tests.

4. We feel entitled to assume that dominant lethal mutations originate mainly from chromosome aberrations (ROHRBORN et al., 1971). The above immediately raises the question whether caffeine could induce genes mutations in mammals and in man. In the course of an extremely time-consuming test aimed at the induction of genetic mutations, LYON at al. found a single mutation only among the 32 463 progeny of caffeine-treated male mice. This corresponds approximately to the spontaneous mutation rate and leads to no conclusions concerning the induction of genetic mutations by caffeine. Using the same testing system, the so-called "specific locus-test", RUSSEL et al. (1968) found "that there is no evidence that caffeine in these experiments has had any effect at all on the repair process." LEGATOR (1970) found in a point-mutation test--the so-called host-mediated essay--that caffeine is not mutagenic. Accordingly, it can be hoped that the question of caffeine -induced point-mutations in mammals and in man as well is not highly significant, despite the fact that--at least in the immediate future--definitely negative evidence will not be obtainable.

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7.

Aus dem Institut für Anthropologie und Humangenetik der Universität Heidelberg (Direktor: Professor Dr. Friedrich Vogel)

Mutagenitätsuntersuchungen an Mäusen nach chronischer Behandlung mit Coffein*)

Von G. Röhrborn

Mit 10 Tabellen

Einleitung

Coffein gehört zu den Substanzen in der menschlichen Umwelt, die von einem großen Teil der Bevölkerung über einen längeren Zeitraum hinweg eingenommen werden. Gerade für solche Substanzen ist die Kenntnis mutagener Wirkung sehr wichtig. Entsprechend zahlreich sind auch die Mutagenitätsuntersuchungen mit Coffein. So berichtete bereits 1929 *Mainx* über eine Coffein-induzierte Mitosehemmung bei Tradescantia pillosa. *Kihlman* und *Levan* beobachteten 1949 einen ähnlichen Effekt bei Allium cepa. Darüber hinaus fanden sie eine Coffein-induzierte Hemmung der Zellwandbildung und Chromosomenaberrationen. Bei Escherichia coli beobachteten *Demerec* et al. (1951), *Gezelius* und *Fries* (1952), *Greer* (1958), *Novick* (1956), *Kubitschek* und *Bendigkeit* (1964) mutagene Wirkung von Coffein. Bei Pilzen konnten *Fries* und *Kihlman* (1948) mutagene Wirkung von Coffein nachweisen. Die Resultate bei Drosophila sind widersprüchlich. So fand *Andrew* (1959) eine schwache mutagene Aktivität von Coffein, konnte jedoch keine spezifisch sensiblen Stadien des Spermatogenesezyklus finden. Wenige Jahre später fanden *Yanders* und *Seaton* unter ähnlichen Versuchsbedingungen keine mutagene Wirkung von Coffein bei Drosophila. Untersuchungen von *Alderson* und *Khan* (1967) an Drosophila fielen ebenfalls negativ aus. Dagegen beobachteten *Ostertag* und *Haake* (1966) mutagene Effekte nach Verfütterung von Coffein an Drosophila-Larven. *Mittler* und Mitarb. (1967) berichteten ferner über einen Anstieg der Nondisjunction-Rate nach Coffein-Behandlung von Drosophila. Dagegen sprechen wiederum die Befunde von *Clark* und *Clark* (1968), die einen Rückgang der Nondisjunction-Häufigkeit beobachteten und darüber hinaus keinen Hinweis für die Induktion X-chromosomaler rezessiver Letalmutationen bei Drosophila fanden. Auf der anderen Seite fanden diese Autoren Hinweise für einen erhöhten Chromosomenverlust in den Coffein-Serien.

In HeLa-Zellkulturen und Lymphozytenkulturen fanden *Ostertag* und Mitarb. Coffein-induzierte Chromosomenbrüche, jedoch nur wenig Reunionsfiguren, d. h. Chromosomenmutationen im engeren Sinn.

Zur Beurteilung der möglichen mutagenen Wirkung einer Verbindung beim Menschen ist die Berücksichtigung des Säugetierstoffwechsels cum grano salis von besonderer Bedeutung. Akute Behandlung mit hohen Coffeindosen bei Mäusen ließ weder im dominanten Letaltest (*Adler*, 1969; *Epstein*, 1970) noch bei der zytogenetischen Untersuchung von Spermato-

*) Mit Unterstützung der Deutschen Forschungsgemeinschaft

zyten auf Chromosomenaberrationen (*Adler*, 1966) mutagene Wirkung erkennen. Da Coffein vom Menschen gewöhnlich chronisch aufgenommen wird, fehlte es auch nicht an Versuchen, mutagene Wirkung durch chronische Coffein-Behandlung festzustellen. Die Befunde sind jedoch nicht eindeutig und zumeist negativ (*Bluhm*, 1924; *Cattanach*, 1962, 1964; *Eichler* et al., 1932; *Kuhlmann*, 1967; *Kuhlmann* et al., 1968; *Lyon* et al., 1962; *Stieve*, 1928).

Das Hauptanliegen der vorliegenden Untersuchung war, eine umfangreiche Mutagenitätsstudie nach einer Langzeitbehandlung mit coffeinhaltigem Trinkwasser in niedrigen Dosisbereichen durchzuführen, da sehr wahrscheinlich Schwellenwerteffekte in der chemischen Mutagenese eine bedeutsame Rolle spielen.

Material und Methode

Wir behandelten männliche Mäuse des C3H-Inzuchtstammes und (101xC3H)-F_1-Hybriden mit drei verschiedenen Coffein-Konzentrationen im Trinkwasser. Die Behandlung wurde im Alter von 3 Wochen begonnen. Alle Tiere entstammten unserer eigenen Zucht und wurden in völlig klimatisierten Tierräumen bei 22° C und einer Luftfeuchtigkeit von 50–55 % gehalten. Die parallel laufenden Kontrollen erhielten nur Leitungswasser als Trinkwasser.

Eine wesentlich kleinere Gruppe von C57BL-Mäusen, die mir von Dr. *Kuhlmann* im Alter von etwa 6 Wochen zur Verfügung gestellt wurden, erhielt eine hohe Coffein-Konzentration als Trinkwasser. Darüber hinaus gaben wir auch einer Gruppe von hauseigenen C3H-Inzucht-♂♂ die gleiche hohe Coffein-Konzentration wie den C57BL-Tieren. Bis zu einem Behandlungszeitraum von 245 bzw. 246 oder in einem Fall sogar 351 Tagen wurden die aufgenommenen Trinkwassermengen und das Körpergewicht der Tiere gemessen. Die gefundenen Werte sind in Tab. 1 zusammengefaßt. Danach wurden die beiden niedrigen, physiologischen Konzentrationen den C3H- und den (101xC3H)F_1-♂♂ bis zu einer Gesamtdauer von 550 Tagen weiter verabreicht.

Während der Behandlung befanden sich jeweils 3 ♂♂ in einem Käfig vom Typ II (Altromin). Wie aus Tab. 1 hervorgeht, nahmen die mit einer 0,0023%igen Coffein-Lösung behandelten Mäuse der Stämme C3H und (101xC3H) F_1 täglich etwa 3,4–3,6 mg Coffein pro kg Körpergewicht auf. Das entspricht ungefähr dem Coffeingehalt von 2–3 Tassen Kaffee. In der nächsthöheren Coffein-Serie (0,01 %) nahmen die C3H-♂♂ im Durchschnitt 14,9 und die (101xC3H)F_1-♂♂ im Durchschnitt 17,3 mg Coffein pro kg Körpergewicht auf. Das entspricht mehr als 10 Tassen Kaffee pro Tag. Entsprechend den Richtlinien der Association of Food and Drug Officials of the United States (1959) kann diese Dosis bei der Maus noch mit der von Menschen aufgenommenen Tagesdosis verglichen werden. Unvergleichbar hoch ist dagegen die 0,5%ige Coffein-Konzentration. Diese Überdosierung wurde absichtlich gewählt, um bei einem negativen Versuchsausgang in der Beurteilung der Versuchsergebnisse sicherer sein zu können. Ein verändertes Trinkverhalten durch den Coffeingehalt des Trinkwassers wurde in den beiden physiologischen Konzentrationen nicht beobachtet, wie aus Tab. 1 hervorgeht. Lediglich in der höchsten, „unphysiologischen" Coffein-Konzentration war die Trinkwasseraufnahme durch den Coffeingehalt reduziert. Auf eine Korrektur der Trinkwasserzufuhr wurde in diesem Überdosierungsversuch absichtlich verzichtet, um auch toxische Nebeneffekte, die die Auslösung von dominanten Letalmutationen durch Coffein begünstigen könnten, mit zu erfassen.

In bestimmten Zeitabschnitten nach der Behandlung wurden die behandelten und unbehandelten ♂♂ mit 10–12 Wochen alten unbehandelten, virginen ♀♀ des jeweils gleichen Stammes gepaart. Etwa am 15. Tag der Trächtigkeit wurden die

Tab. 1. Durchschnittliche tägliche Aufnahme verschiedener Coffein-Konzentrationen im Trinkwasser männlicher Mäuse

Stamm	% Coffein-Konzentration	n ♀♀	Coffein-Aufnahme in Tagen	durchschnittliche Aufnahme cc/g Körpergewicht pro Tag	mg Coffein / kg Körpergewicht pro Tag
(101 × C3H)F_1	0,0023	96	245	0,1475	3,39
(101 × C3H)F_1	Kontrolle	39	245	0,1359	–
(101 × C3H)F_1	0,01	18	246	0,1723	17,23
(101 × C3H)F_1	Kontrolle	6	246	0,1225	–
C3H	0,0023	90	245	0,1555	3,58
C3H	Kontrolle	24	245	0,1355	–
C3H	0,01	81	246	0,1492	14,92
C3H	Kontrolle	18	246	0,1292	–
C3H	0,5	15	351	0,0610	304,92
C3H	Kontrolle	6	351	0,0997	–
C57BL	0,5	7	245	0,1029	514,33
C57BL	Kontrolle	4	245	0,1261	–

♀♀ seziert, die Corpora lutea graviditatis gezählt und die Zahl der lebenden und toten Implantate nach Öffnung des Uterus bestimmt. Entsprechend unseren früheren Ausführungen (*Röhrborn*, 1968, 1970 a) betrachten wir nach Behandlung männlicher Tiere insbesondere einen Anstieg der Rate toter Implantate als Kriterium für induzierte dominante Letalmutationen. Präimplantative Eiverluste allein halten wir dagegen nicht für ein alleingültiges und ausreichendes Kriterium für dominante Letalmutationen, da sie u. a. auch auf hochgradige Oligospermien oder Bewegungsinaktivierung der Spermien zurückzuführen sein könnten.

Die statistische Berechnung der Versuchsergebnisse erfolgte mit der χ^2-Methode.

Ergebnisse

1. *Behandlung von C57BL-♂♂*

In einem kleinen orientierenden Versuch zur Empfindlichkeit von C57BL-Mäusen behandelten wir 11 C57BL-♂♂ mit einer extrem hohen 0,5%igen Coffein-Lösung im Trinkwasser. Zur Kontrolle erhielten 4 C57BL-♂♂ gleichzeitig Leitungswasser. Wie bereits erwähnt, begann in diesem Fall die Behandlung im Alter von 6 Wochen, nachdem wir die Tiere von Dr. *Kuhlmann* erhalten hatten. 161 Tage später unterbrachen wir die Behandlung für die Dauer von 8 Wochen und paarten jedes ♂ mit 3 virginen 10–12 Wochen alten (101 × C3H)F_1-♀♀ pro Woche. Die Ergebnisse des damit verbundenen dominanten Letaltests zeigt Tab. 2.

Wie aus Tab. 2 hervorgeht, waren nach 161 Tagen Behandlung die präimplantativen Eiverluste in der Coffein-Serie gegenüber der Kontrolle erhöht. Der Unterschied war statistisch signifikant (P ~ 0,0027). Auf der

Tab. 2. Versuchsergebnisse mit der dominanten Letalmutationsmethode nach chronischer Behandlung von C57BL-♂♂[1]) mit einer extrem hohen Coffein-Konzentration im Trinkwasser

Trinkwasser	Behandl. in Tagen	n befr. ♀♀	n Corp. lut.	n Impl.	n Impl. pro ♀	n leb. Embr.	n leb. Embr. pro ♀	% PE[2]) probability		n TI[3])	% TI[4]) probability	
0,5% Coffein	161	62	607	465	7,50	428	6,90	23,4	P ~ 0,0027	37	8,0	P ~ 0,8
Kontrolle	161	15	148	130	8,67	119	7,93	12,2		11	8,5	
0,5% Coffein	245	48	444	375	7,81	350	7,29	15,5	P ~ 0,26	25	6,7	P ~ 0,74
Kontrolle	245	10	99	88	8,80	83	8,30	11,1		5	5,7	

[1]) mit 10–12 Wochen alten (101 × C3H)F_1-♀♀ verpaart
[2]) PE = präimplantativer Eiverlust
[3]) TI = tote Implantate
[4]) in % der Gesamtzahl der Implantate

anderen Seite waren die Raten toter Implantate (bezogen auf die Gesamtzahl der Implantate) in der Coffein-Serie sogar ein wenig niedriger als in den mitlaufenden Kontrollen.

Nach dieser 8wöchigen Auskreuzung wurden dieselben Tiere bis zu einer Gesamtdauer von 245 Tagen mit derselben Coffein-Konzentration weiter behandelt. Als Kontrolle wurden dieselben ♂♂ wie bisher benutzt. Danach erfolgte die Auskreuzung wieder mit 10–12 Wochen alten (101xC3H)-F_1-♀♀. Die Ergebnisse sind in Tab. 2, untere Reihe, dargestellt. Es zeigte sich, daß weder die präimplantativen Eiverluste noch die Raten toter Implantate in der Coffein-Serie über den Kontrollwerten lagen.

2. Behandlung von C3H-♂♂

Wie aus Tab. 1 hervorgeht, behandelten wir 90 C3H-♂♂ mit einer 0,0023%igen Coffein-Konzentration, 81 C3H-♂♂ mit einer 0,01%igen Coffein-Konzentration und 14 C3H-♂♂ mit einer 0,5%igen Coffein-Konzentration. Nach bestimmten Zeitabständen wurden die ♂♂ mit 10–12 Wochen alten, unbehandelten, virginen ♀♀ des gleichen Stammes ausgekreuzt. In dieser Zeit wurde die Behandlung ausgesetzt. Die Paarungsdauer betrug in den beiden „physiologischen" Coffein-Bereichen nur 2 Wochen, da eine große Anzahl von ♂♂ zur Verfügung stand. Die 0,5 %-Coffein-Serie wurde nach 146 Tagen für 3 Wochen und nach 351 Tagen für die Dauer von 8 Wochen verpaart, um das Material zu vergrößern, da in dieser Serie nur eine geringe Anzahl von behandelten ♂♂ zur Verfügung stand.

In Tab. 3 sind die Werte der einzelnen Wochen zusammengefaßt, nachdem sich in einem Heterogenitätstest herausgestellt hatte, daß die Ergebnisse der einzelnen Wochen nicht signifikant voneinander differieren ($P > 0{,}03$ nach *Brand-Snedecor*). Wie aus Tab. 3 hervorgeht, liegt der einzige auffällige Unterschied zwischen Versuch und Kontrolle in der 0,01%igen Coffein-Serie nach 91 Tagen Behandlung. Der P-Wert für die Unterschiede in den Raten toter Implantate beträgt 0,015. Wie bereits 1968 ausgeführt (*Röhrborn*, 1968), sollte man jedoch in Untersuchungen zur Auslösung von dominanten Letalmutationen nur P-Werte, die kleiner als 0,0027 sind, als Kriterien für signifikante Differenzen zwischen Versuch und Kontrolle werten. Daher wurde von vornherein vermutet, daß dieser Unterschied zufälligen Charakter trägt. Dieser Eindruck wird dadurch verstärkt, daß der P-Wert nach 245 Tagen Behandlung größer ($\sim$ 0,20) wird, was allerdings mit der relativ geringen Anzahl der Implantate zusammenhängen könnte. Darüber hinaus lieferten weder der Versuch mit der niedrigsten Konzentration (0,0023 %) noch der mit der höchsten Konzentration (0,5 %) deutliche Hinweise für die Induktion von dominanten Letalmutationen. Im Gegenteil, die P-Werte für die toten Implantate, die in Tab. 3 aufgeführt sind, deuten eher auf Homogenität von Versuch und Kontrolle hin.

Um das Problem abzuklären, führten wir jedoch mit den beiden niedrigen Coffein-Konzentrationen die Behandlung bis zu einer Gesamtdauer von 550 Tagen weiter. Nach dieser Zeit wurden die behandelten ♂♂ und ihre Kontrollen jeweils 3 Wochen lang wieder mit 10–12 Wochen alten virginen ♀♀ des gleichen Stammes ausgekreuzt und die Raten der präimplantativen Eiverluste und der toten Implantate bestimmt.

Tab. 3. Versuchsergebnisse mit der dominanten Letalmutationsmethode nach chronischer Behandlung von C3H-♂♂ mit 3 Coffein-Dosen

Versuch	Behandl.-dauer	n c.l.	n Impl.	% PE[1])	n TI[2])	% TI[3])	probability	% PE + TI[4])	probability
0,0023% Coffein	245	877	802	8,6	99	12,3	~ 0,36	19,8	~ 0,66
Kontrolle	245	242	219	9,5	22	10,0		18,6	
0,01% Coffein	91	2163	1911	11,7	268	14,0	~ 0,015	24,0	~ 0,03
Kontrolle	91	649	577	11,1	58	10,1		20,0	
0,01% Coffein	245	679	603	11,2	76	12,6	~ 0,20	22,4	~ 0,07
Kontrolle	244	130	120	7,7	10	8,3		15,4	
0,5% Coffein	146	820	741	9,6	136	18,4	~ 0,68	26,2	~ 0,44
Kontrolle	146	335	307	8,4	53	17,3		24,2	
0,5% Coffein	351	1460	1292	11,5	177	13,7	~ 0,90	22,1	~ 0,2
Kontrolle	351	796	727	8,7	101	13,9		21,4	
						$\varkappa^2 = 8{,}92$		$\varkappa^2 = 9{,}93$	
						P [5] ~ 0,10		P [5] ~ 0,08	

[1]) Präimplantativer Eiverlust in % der Corpora lutea-Zahlen
[2]) TI = tote Implantate
[3]) in % der Gesamtzahl der Implantate
[4]) in % der Corpora lutea-Zahl

Tab. 4. Prä- und postimplantative Eiverluste nach 550 Tagen Coffein-Behandlung von C3H-♂♂

	n befr. ♀♀	n c.l.	n Impl.	% PE	probability	% TI	probability
0,0023% Coffein	87	907	755	16,9	> 0,3	11,0	< 0,6
Kontrolle	19	188	163	13,3		12,3	
0,01% Coffein	46	419	355	12,9	> 0,8	13,8	> 0,2
Kontrolle	8	75	63	10,7		19,0	

Wie aus Tab. 4 hervorgeht, lagen zwar in beiden Coffein-Serien die präimplantativen Eiverluste etwas über den Kontrollen, dafür waren aber die Raten toter Implantate in den Coffein-Serien niedriger als in den Kontrollen; die Unterschiede sind in keiner Richtung signifikant. Die Homogenität zwischen den Versuchs- und Kontrollserien wird noch deutlicher, wenn man die Anzahl der lebenden Embryonen pro befruchtetes ♀ vergleicht (Tab. 5).

Tab. 5

Versuch	n lebende Embryonen pro befruchtetes C3H-♀
0,0023% Coffein	
550 Tage	7,72
Kontrolle	7,53
0,01% Coffein	
550 Tage	6,65
Kontrolle	6,38

Da die durchschnittliche Anzahl der lebenden Embryonen pro fertiles ♀ in beiden Coffein-Serien sogar noch höher ist als in den mitlaufenden Kontrollen, spricht dies gegen eine Induktion dominanter Letalmutationen durch die Behandlung.

3. Behandlung von (101×C3H)F_1-♂♂

In der 0,01%igen Coffein-Serie wurden die behandelten Tiere und ihre Kontrollen nach 105 Tagen für 1 Woche und nach 246 Tagen für 8 Wochen mit jeweils 3 virginen ♀♀ im Alter von 10–12 Wochen vom gleichen Typ ausgekreuzt. Die Ergebnisse der einzelnen Wochen wurden wiederum zusammengefaßt. In der 0,0023 %-Coffein-Serie wurden die Tiere nach 250-tägiger Behandlung für die Dauer von 2 Wochen mit 10–12 Wochen alten virginen ♀♀ des gleichen Stammes verpaart. Da sich die Ergebnisse der ein-

zelnen Wochen wiederum nicht als heterogen erwiesen, sind sie in Tab. 6 in den einzelnen Experimentalserien und ihren Kontrollen zusammengefaßt.

Tab. 6. Versuchsergebnisse mit der dominanten Letalmutationsmethode nach chronischer Behandlung von (101 × C3H) F_1-♂♂)[1]) mit niedrigen Coffein-Dosen

Versuch	Behandlungsdauer in Tagen	n befr. ♀♀	n c.l.	n Impl.	n Impl. pro befr. ♀	n leb. Embr.	n leb. Embr. pro ♀
0,0023% Coffein	245	171	1794	1569	9,18	1461	8,54
Kontrolle	245	71	716	645	9,08	599	8,44
0,01% Coffein	105	39	410	364	9,33	336	8,62
Kontrolle	105	13	125	116	8,92	106	8,15
0,01% Coffein	246	257	2509	2288	8,90	2113	8,22
Kontrolle	246	93	890	804	8,65	748	8,04

[1]) Zu Beginn der Behandlung waren alle ♂♂ 3 Wochen alt. Nach der Behandlung wurden die ♂♂ mit 10–12 Wochen alten (101 × C3H) F_1-♀♀ gepaart.

Tab. 6 zeigt deutlich folgende zwei Fakten:

1. Die Anzahl der Implantate pro befruchtetes ♀ ist in jeder Coffein-Serie sogar höher als in der entsprechenden Kontrolle. Das bedeutet, daß kein Coffein-induzierter, präimplantativer Eiverlust aufgetreten ist.

2. Auch die Anzahl der lebenden Embryonen pro befruchtetes ♀ ist in jeder Coffein-Serie etwas höher als in der entsprechenden Kontrolle. Dies zeigt klar, daß auch keine Coffein-induzierten toten Implantate durch die Behandlung der ♂♂ auftraten.

In Mutagenitätsexperimenten kann man zwei verschiedenen Irrtumstypen unterliegen: Der erste ist der, eine Verbindung mutagen zu nennen, wenn keine genügende Absicherung gegeben ist ($P > 0{,}0027$ für dominante Letalexperimente). Die zweite Irrtumsmöglichkeit ist die, eine Verbindung nicht-mutagen zu nennen, wenn in Wirklichkeit eine schwache Mutagenität vorhanden ist. Abgesehen von der fehlenden Signifikanz für induzierte dominante Letalmutationen haben wir uns deshalb gefragt, wie sicher der so überzeugend scheinende negative Befund in diesen Versuchen ist. Nach dem Prüfverfahren von *Strobel* und *Vogel* (1958) konnten wir zeigen, daß die Experimente mit der höchsten Dosis allein schon einen Coffein-induzierten Anstieg der prä- und postimplantativen Eiverlustraten um mehr als den Faktor 1,16 ausschließen. (95 % Vertrauensgrenze). – Nichtsdestoweniger behandelten wir auch diese ♂♂ weiter bis zu einer Gesamtdauer von 550 Tagen. Danach verpaarten wir die Tiere der 0,0023%igen Serie für die Dauer von 3 Wochen und die der 0,01%igen Serie aus Gründen der Materialvergrößerung für die Dauer von 8 Wochen wieder mit unbehandelten 10–12 Wochen alten virginen ♀♀ des gleichen Typs und faßten die einzelnen Serien nach einer Heterogenitätsprüfung zusammen (Tab. 7).

Tab. 7. Prä- und postimplantative Eiverluste nach 550 Tagen Coffein-Behandlung von (101 × C3H)F_1-♂♂

	n befr. ♀♀	n c.l.	n Impl.	% PE	probability	% TI	probability
0,0023% Coffein	135	1323	1190	10,1	> 0,2	7,7	> 0,8
Kontrolle	50	498	437	12,2		8,0	
0,01% Coffein	29	274	245	10,6	~ 0,7	11,4	0,05–0,1
Kontrolle	8	77	70	9,1		4,3	

Wie aus Tab. 7 hervorgeht, finden sich keine deutlichen Hinweise für induzierte dominante Letalmutationen. Dieser Eindruck wird wiederum deutlicher, wenn man die in Tab. 8 aufgeführten durchschnittlichen Zahlen der lebenden Embryonen pro befruchtetes ♀ vergleicht.

Tab. 8

Versuch	n lebende Embryonen pro befruchtetes (101 × C3H)F_1-♀
0,0023% Coffein	
550 Tage	8,1
Kontrolle	8,0
0,01% Coffein	
550 Tage	7,5
Kontrolle	8,4

4. Behandlung von C3H- und (101 × C3H)F_1-♀♀

Da möglicherweise weibliche Individuen gegenüber der Induktion von dominanten Letalmutationen durch Coffein empfindlicher sein könnten als männliche, behandelten wir weibliche Mäuse für die Dauer von 43 Wochen mit 0,01%igem Coffein-Trinkwasser. Die parallel laufenden Kontrollen erhielten normales Leitungswasser. Die Behandlung begann im Alter von 3–4 Wochen. Nach Absetzen der Behandlung wurden die Tiere mit unbehandelten 10–12 Wochen alten ♂♂ verpaart. Die Ergebnisse sind in Tab. 9 dargestellt. Aus dieser Tabelle geht klar hervor, daß in beiden Stämmen sowohl in den Kontrollen als auch in den Experimenten die Raten der präimplantativen Eiverluste und der toten Implantate beträchtlich höher lagen als die in den anderen Tabellen aufgeführten Werte in den Versuchen und Kontrollen. Die einzige Erklärung für diesen Effekt ist, daß die hohen Raten präimplantativer Eiverluste und toter Implantate durch das hohe mütterliche Alter bedingt sind. Gerade diese Befunde zeigen deutlich, wie wichtig homogene Altersgruppen in Versuch und Kontrolle bei derartigen Experimenten sind.

Tab. 9. Präimplantativer Eiverlust und tote Implantate nach chronischer Behandlung weiblicher Mäuse mit einer 0,01%igen Coffein-Konzentration im Trinkwasser für die Dauer von 43 Wochen (Alter der Tiere bei Beginn der Behandlung: 3–4 Wochen)

Stamm	Paarungszeit in Wochen nach Behandlung	n c.l.		n Impl.		% PE		% TI	
		V	K	V	K	V	K	V	K
C3H	1.	122	58	70	32	42,6	44,8	32,9	34,4
(101 × C3H)F_1	1.	480	243	321	152	33,1	37,4	48,3	44,7
(101 × C3H)F_1	2.	427	120	261	88	38,9	26,7	57,1	70,5

V = Versuch (0,01% Coffein 43 Wochen lang)
K = Kontrolle

In einem kleinen orientierenden Versuch prüften wir ferner die Frage, ob die akute Behandlung mit einer hohen Coffein-Dosis zu einem Coffein-induzierten Anstieg der präimplantativen Eiverluste und/oder der toten Implantate führt. Die Ergebnisse sind in Tab. 10 dargestellt.

Tab. 10. Vorläufige Versuchsergebnisse mit der dominanten Letalmutationsmethode nach einmaliger intraperitonealer Injektion von 0,25 g/kg Coffein bei (101 × C3H)F_1-♀♀[1])

Paarungstage nach Injektion	n befr. ♀♀		n c.l.		n Impl.		% PE		n tote Impl.		% tote Impl.	
	V[2])	K[2])	V	K	V	K	V	K	V	K	V	K
0– 1	8	5	80	55	74	49	7,5	10,9	7	3	9,5	6,1
2– 8	21	9	197	85	173	80	12,2	5,9	18	4	10,4	5,0
9–15	33	10	302	97	274	89	9,3	8,2	27	12	9,9	13,5
16–22	36	10	335	107	294	100	12,2	6,5	26	7	8,8	7,0
23–29	39	9	391	90	351	79	10,2	12,2	37	5	10,5	6,3
30–36	14	9	142	96	124	86	12,7	10,4	6	13	4,8	15,1
Σ 0–36	151	52	1447	530	1290	483	10,9	8,9	121	44	9,4	9,1

[1]) Alter der Behandlung: 10 Wochen ± 2 Tage; Alter der (101 × C3H)F_1-♂♂ zur Zeit der Paarung: 10–11 Wochen.
[2]) V = Coffein-Serie; K = Kontrolle

Da sich in Untersuchungen mit Endoxan besonders unter den am 1. Tag nach Absetzen der Behandlung befruchteten ♀♀ hohe Raten induzierter toter Implantate fanden (*Röhrborn*, 1970 b), wurde dieser Tag speziell analysiert (erste Reihe). Für die weiteren Analysen wurden die Werte wochenweise zusammengefaßt. Weder in den einzelnen Wochen noch insgesamt zeigten sich signifikante Unterschiede zwischen Versuch und Kontrolle in bezug auf die Raten toter Implantate. Auch lagen die Raten der präimplantativen Eiverluste in der Coffein-Serie nur unwesentlich höher

als in der Kontrolle. Das bedeutet, daß wir auch mit der akuten Behandlung weiblicher Mäuse keine deutlichen Hinweise für die Induktion dominanter Letalmutationen gewinnen konnten.

Diskussion

Dominante Letalmutationen können sich sowohl in präimplantativen Eiverlusten als auch in toten Implantaten äußern. Nach Behandlung von männlichen Tieren können präimplantative Eiverluste jedoch auch durch Oligospermien und andere nichtmutative Prozesse ausgelöst werden. Daher sind u. E. tote Implantate das wesentlich bessere Kriterium für dominante Letalmutationen. Schon aus diesem Grunde können die nach 161 Tagen Behandlung von C57BL-♂♂ mit 0,05 %iger Coffein-Lösung als Trinkwasser aufgetretenen präimplantativen Eiverluste nicht als beweiskräftig für die Induktion dominanter Letalmutationen angesehen werden, zumal die Raten toter Implantate keine signifikanten Unterschiede zwischen Versuch und Kontrolle zeigten. In früheren Untersuchungen mit Trypaflavin und Hexamethylentetramin (*Baldermann* et al., 1967) hatte sich gezeigt, daß sich eine schwache Mutagenität einer Verbindung nur in den Raten toter Implantate manifestiert. Dies ist dadurch zu erklären, daß einzelne Letaltreffer pro Keimzelle zu einer relativ späten embryonalen Sterblichkeit, d. h. zu toten Implantaten führen. Starke Mutagene führen dagegen außer zu erhöhten Raten toter Implantate auch zu hohen präimplantativen Eiverlusten, was durch die Tatsache erklärt werden könnte, daß in den letztgenannten Fällen mehrere Letaltreffer pro Gamete vorliegen. Mit anderen Worten: je mehr Letaltreffer pro Keimzelle, desto früher stirbt der Embryo ab. Es erscheint deshalb unwahrscheinlich, daß Coffein gerade entgegengesetzte Effekte verursachen sollte, d. h. daß sich die Coffein-induzierten dominanten Letalmutationen praktisch ausschließlich in der präimplantativen Phase auswirken sollten. Darüber hinaus sei daran erinnert, daß nach Fortsetzen der Behandlung die präimplantative Eiverlustrate zwischen Versuch und Kontrolle nicht mehr signifikant verschieden war.

In den Versuchen mit C3H- und (101xC3H)F_1-♂♂ fanden wir ebenfalls keine signifikante Induktion dominanter Letalmutationen durch Coffein. Insbesondere die Versuche an hybriden Mäusen (Tab. 6) schließen eine Induktion dominanter Letalmutationen durch Coffein mit einem recht guten Grad an Sicherheit aus (siehe Erläuterungen zu Tab. 6). Darüber hinaus sprechen auch die Ergebnisse nach 550tägiger Behandlung gegen eine Coffein-induzierte dominante Letalität in diesen beiden „physiologischen" Konzentrationsbereichen. Unsere Versuchsergebnisse stehen in guter Übereinstimmung mit den Coffein-Untersuchungen von *Lyon* et al. (1962) und *Adler* (1969). Darüber hinaus lieferte auch die zytogenetische Untersuchung von *Adler* (1966) nach akuter Behandlung sowie von *Adler* und *Röhrborn* (1969) nach chronischer Behandlung keine deutlichen Hinweise für Mutagenität. Das Material von *Kuhlmann* et al. (1968) erscheint bezüglich der Gesamtzahl der Implantate und der Zahl der toten Implantate in den einzelnen Konzentrationsbereichen und vor allem in der Kontrolle (52 Implantate bei 8 ♀♀) zu klein, um als beweiskräftig für die Induktion dominanter Letalmutationen durch Coffein zu gelten. *Adler* (1970),

die diese Arbeit ausführlich diskutiert, kritisiert ferner richtig, daß die Kontrolle nicht parallel zu den einzelnen Experimenten lief. Darüber hinaus führt *Adler* an, daß die Experimentalserien von *Kuhlmann* auch jene ♀♀ einschließen, die nur tote Implantate hatten. Dies könnte u. E. eher auf einen Infekt des Muttertieres als auf eine Induktion dominanter Letalmutationen in den Keimzellen des Vatertiers beruhen.

Unsere negativen Resultate stehen darüber hinaus in guter Übereinstimmung mit den Befunden von *Vogel* et al. (1966), die in einer Erhebung beim Menschen keine Hinweise für eine mutagene Wirkung von Coffein fanden. Daher lassen unsere Untersuchungen und die von *Vogel* et al. hoffen, daß zumindest in niedrigen Konzentrationsbereichen Coffein keine oder nur eine schwache mutagene Wirkung bei Säuger und Mensch entfaltet. Dennoch bleiben verschiedene Fragen offen:

1. Sind die von uns benutzten Stämme besonders unempfindlich gegenüber einer induzierten Coffein-Mutagenese? Dieses Argument wird etwas abgeschwächt dadurch, daß *Cattanach* (1962, 1964), *Lyon* et al. (1962) und *Epstein* (1970) ebenfalls keine signifikanten Hinweise für Coffein-induzierte Mutationen in Experimenten mit anderen Mäusestämmen fanden.

2. Sind Mäuse besonders unempfindlich bezüglich Coffein-Mutagenese? Dies Problem sollte durch Verwendung anderer Säuger-Spezies geklärt werden.

3. Wie steht es mit synergistischen Effekten? *Witkin* (1958), *Lieb* (1961) und *Lumb* et al. (1968) fanden z .B. „Dark-repair"-Hemmung ultraviolett-induzierter Mutationen bei Bakterien durch Coffein. *Harm* (1967) erhielt ähnliche Ergebnisse bezüglich des Überlebens von E. coli nach UV-Bestrahlung in Gegenwart von Coffein, und *Grigg* (1968) entwickelte ein Modell von Coffein-induziertem Tod von E. coli durch Chromosomenbrüche, die unter der Hemmung von Repair-Enzymen zustande kamen. *Wragg* et al. (1967) beobachteten ferner die Hemmung von DNS-Polymerase-Aktivität durch Coffein in einer Säugerzellkultur bei Konzentrationen über 200 μg/ml. *Adler* und *Schöneich* (1967) berichteten über vermehrtes Vorkommen von Chromosomenbrüchen in S_2-Sarkomen von Mäusen nach intraperitonealer Injektion von reinem Sauerstoff und hohen Coffein-Dosen. Coffein und Sauerstoff, jeweils allein verabreicht, zeigten keinen Effekt. Andererseits fand *Clarke* (1968) bei Schizosaccharomyces pombe, daß „caffeine is without any obvious effect on the lethality of met$^+$ reversions induced by nitrous acid or nitrosomethylurethane treatments. This suggests that the caffeine-sensitive dark repair system is only specific for some types of lesion." Des weiteren fand *Wolff* (1968) mit der Röntgen-splitting-Technik bei Vicia faba keinen Hinweis dafür, daß Coffein den Repair-Mechanismus bei Röntgen-induzierten Chromosomenbrüchen hemmt. *Cleaver* (persönl. Mitteilg.) fand ebenfalls keine Hemmung von Repair-Prozessen in Säugerzellkulturen. Schließlich konnte *Epstein* (1970) bei Mäusen keine synergistischen Effekte von Coffein und Methylmethansulfonat bzw. Röntgenstrahlen in dominanten Letaltests nachweisen.

4. Wie wir annehmen dürfen, beruhen dominante Letalmutationen im wesentlichen auf Chromosomenaberrationen (*Röhrborn* et al., 1971). Dies wirft sofort die Frage auf, ob Coffein nicht Genmutationen bei Säuger und

Mensch induzieren könnte. *Lyon* et al. fanden in einem sehr zeitraubenden Versuch zur Induktion von Genmutationen unter 32 463 Nachkommen Coffein-behandelter männlicher Mäuse nur eine Mutation; das entspricht etwa der spontanen Mutationsrate und läßt keine Schlüsse auf die Induktion von Genmutationen durch Coffein zu. Mit der gleichen Versuchsanordnung, dem sog. Specific locus-Test fanden *Russel* et al. 1968, „that there is no evidence that caffeine in these experiments has had any effect at all on the repair process". *Legator* (1970) fand Coffein in einem Punktmutationstest, dem sog. host-mediated assay, nicht mutagen. Dies läßt hoffen, daß auch der Frage Coffein-induzierter Punktmutationen bei Säuger und Mensch keine große Bedeutung zukommt, obwohl zumindest in nächster Zeit ein endgültiger negativer Beweis nicht zu führen sein wird.

Zusammenfassung

Männliche C57BL-, C3H- und (101xC3H)F_1-Mäuse wurden über einen sehr langen Zeitraum chronisch mit verschiedenen Coffeindosen behandelt und mit der dominanten Letalmutationsmethode auf Induktion von Mutationen geprüft.

Nach 161 Tagen Behandlung von C57BL-♂♂ mit einer extrem hohen Dosis von 0,5 % Coffein als Trinkwasser fand sich ein signifikanter Anstieg der präimplantativen Eiverluste. Nach 245 Tagen waren die präimplantativen Eiverlustraten in dieser Coffein-Serie wieder auf die Kontroll-Werte abgesunken. Die Behandlung der C3H-♂♂ mit 0,0023 %, 0,01 % bzw. 0,5 % Coffein-Konzentrationen erbrachte keine deutlichen Hinweise für die Induktion dominanter Letalmutationen. Die dominanten Letalmutationsexperimente mit (101xC3H) F_1-Hybriden lieferten ebenfalls keine Hinweise für eine mutagene Wirkung von Coffein. Selbst nach einer Behandlungsdauer von 550 Tagen fanden sich weder beim C3H-Inzuchtstamm noch bei den (101xC3H)F_1-Hybriden Hinweise für induzierte dominante Letalmutationen. Darüber hinaus erbrachte auch die Behandlung weiblicher Mäuse keine Anhaltspunkte für coffeininduzierte dominante Letalmutationen.

Summary

Male C57BL, C3H and (101xC3H)F_1 mice were exposed to long-time oral treatment with various caffeine doses and investigated for induced mutations by means of the dominant lethal method.

After 161 days of treatment with an extremely high caffeine concentration of 0.5 % as drinking water we observed a significant increase of pre-implantation egg loss. After 245 days of treatment, however, the values of the caffeine series again corresponded to those of the controls. Treatment of C3H males with 0.0023 %, 0.01 and 0.5 % caffeine concentrations as drinking water gave no significant evidence for the induction of dominant lethal mutations. In dominant lethal experiments with (101xC3H)F_1 hybrids we could not observe mutagenic effects of caffeine. Even after treatment for 550 days, neither in the C3H inbred strain nor in the (101xC3H)F_1 hybrids induced dominant lethal mutations were found. Furthermore, also by acute treatment of female mice with high caffeine concentrations induction of dominant lethal mutations could not be proven.

Literatur

Adler, I.-D., Humangenetik 3, 82 (1966). – *Adler, I.-D.*, Humangenetik 7, 137 (1969). – *Adler, I.-D.*, The problem of caffeine mutagenicity. In: Chemical Mutagenesis in Mammals and Man, *F. Vogel* and *G. Röhrborn*, Eds. (Berlin-Heidelberg-New York 1970). – *Adler, I.-D.* and *G. Röhrborn*, Humangenetik 8,

81 (1969). – *Adler, I.-D.* and *J. Schöneich*, Humangenetik **4**, 374 (1967). – *Alderson, T.* and *A. H. Khan*, Nature **215**, 1080 (1967). – *Andew, L. E.*, Amer. Nat. **92**, 135 (1959). – *Baldermann, K. H., G. Röhrborn* and *T. M. Schroeder*, Humangenetik **4**, 112 (1967). – *Bluhm, A.*, Arch. Rassenbiol. **16**, 1 (1924). – *Brand-Snedecor*, see *E. Weber*, Grundriß der biologischen Statistik, 4. Aufl. (Jena 1961). – *Cattanach, B. M.*, Z. Vererbungsl. **92**, 215 (1962). – *Cattanach, B. M.* In: *Carson* and *Gassner*, Effects of ionizing radiation on the reproductive system. 415–426 (Oxford 1964). – *Clark, A. M.* and *E. G. Clark*, Mutation Res. **6**, 227 (1968). – *Clarke, C. H.*, Mutation Res. **5**, 33. (1968). – *Demerec, M., G. Bertani* and *J. Flint*, Amer. Nat. **85**, 119 (1951). – Food & Drug Officials of the United States, Association of: Appraisal of the safety of chemicals in foods, drugs and cosmetics. (Baltimore 1959). – *Eichler, O.* und *H. Mügge*, Arch. exper. Path. Pharmakol. **8**, 89 (1932). – *Epstein, S. S.*, In: Chemical Mutagenesis in Mammals and Man, *F. Vogel* and *G. Röhrborn*, Eds., (Berlin-Heidelberg-New York 1970). – *Fries, N.* and *B. A. Kihlmann*, Nature (London) **162**, 573 (1948). – *Gezelius, K.* and *N. Fries*, Hereditas **38**, 112 (1952). – *Greer, S. B.*, J. Gen. Microbiol. **18**, 543 (1958). – *Grigg, G. W.*, Molec. Gen. Genetics **102**, 316 (1968). – *Harm, W.*, Mutation Res. **4**, 93 (1967). – *Kihlman, B. A.* and *A. Levan*, Hereditas 35, 109 (1949). – *Kubitschek, H. E.* and *H. E. Bendigkeit*, Mutations Res. **1**, 113 (1964). – *Kuhlmann, W.*, Referat auf der Tagung der Deutschen Gesellschaft für Anthropologie und Humangenetik (Königstein 1967). – *Kuhlmann, W., H.-G. Fromme, E.-M. Hege* and *W. Ostertag*, Cancer Res. **28**, 2375 (1968). – *Legator, M. S.*, The host-mediated assay, a practical procedure for evaluating potential mutagenic agents. In: Chemical Mutagenesis in Mammals and Man, *F. Vogel* and *G. Röhrborn*, Eds., (Berlin-Heidelberg-New York 1970). – *Lieb, M.*, Z. Vererbungsl. **92**, 416 (1961). – *Lumb, J. R., A. S. Sidcropoulos* and *D. M. Shankel*, Molec. Gen. Genetics **102**, 108 (1968). – *Lyon, M. F., J. S. R. Philip* and *A. G. Searle*, Z. Vererbungsl. **93**, 7 (1962). – *Mainx, F.*, Zool. Jb., Abtg. Allg. Zool., **41**, 553 (1929). – *Mittler, S., J. E. Mittler, A. M. Tonetti* and *M. E. Szymczak*, Mutation Res. **4**, 708 (1967). – *Novick, A.*, Brookhaven Symp. Biol. **8**, 201 (1956). – *Ostertag, W.* and *J. Haake*, Z. Vererbungsl. 98, 299 (1966). – *Röhrborn, G.*, Humangenetik **6**, 345 (1968). – *Röhrborn, G.*, The dominant lethals: Method and cytogenetic examination of early cleavage stages. In: Chemical Mutagenesis in Mammals and Man. *F. Vogel* and *G. Röhrborn*, Eds., (Berlin-Heidelberg-New York 1970 a). – *Röhrborn G.*, The activity of alkylating agents. I. Sensitive mutable stages in spermatogenesis and oogenesis. In: Chemical Mutagenesis in Mammals and Man, *F. Vogel* and *G. Röhrborn*, Eds., (Berlin-Heidelberg-New York 1970 b). – *Röhrborn, G., O. Kühn, I. Hansmann, K. Thon*, Humangenetik **11**, 316 (1971). – *Russel, W. L., R. B. Cumming* and *Elizabeth M. Kelly*, Biol. Div. Ann. Progr. Rept. Dec. **31**, ORNL-4412, pp. 100–101 (1968). – *Stieve, H.*, Z. mikr. anat. Forschg. **15**, 599 (1928). – *Stieve, H.*, Z. mikr. anat. Forschg. **23**, 571 (1931). – *Strobel, D.* und *F. Vogel*, Acta Genet. Stat. Med. **8**, 274 (1958). – *Vogel, F., J. Krüger, M. Kurth* und *T. M. Schroeder*, Humangenetik **2**, 119 (1966). – *Witkin, E. M.*, Post-irradiation metabolism and the timing of ultraviolet induced mutations in bacteria. Proc. 10th Int. Congr. Genetics, Montreal, **1**, 280 (1958). – *Wolff, S.*, Lecture at the Genetics Division of the National Institute of Radiobiological Sciences, Chiba (Japan), Sept. 1968. – *Wragg, J. B., J. V. Carr* and *V. Ross*, J. Cell Biol. **35**, 146 A (1967). – *Yanders, A. F.* and *R. X. Seaton*, Amer. Nat. **46**, 277 (1962).

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THE EXPERIMENTAL PRODUCTION OF ACUTE AND SUBACUTE GASTRIC ULCERS IN CATS BY THE INTRAMUSCULAR INJECTION OF CAFFEINE IN BEESWAX

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INTRODUCTION

Earlier studies on the role of hematogenous gastritis in the pathogenesis of gastric ulcers by Hanke (1) demonstrated that caffeine was capable of producing an acute erosive gastritis in cats with toxic doses. Hanke also observed (2) chronic gastric ulcers after the prolonged daily administration of caffeine sodium salicylate subcutaneously. Grasso (3) was unable to produce any effect upon the gastric mucosa of rabbits with an infusion of coffee by the oral route, but obtained ulcerative gastritis in rabbits when he gave caffeine plus nicotine parenterally. Molteni (4) showed similar erosive and ulcerative lesions in the antrum and pylorus of rats after giving caffeine sodium salicylate over a long time. That caffeine-containing beverages might play a role in the pathogenesis of human peptic ulcers has been implied by various investigators, but the problem has received little attention in the English literature. Very few clinicians restrict the use of coffee in their management of the peptic ulcer patient. The recent report of Judd (5) on the experimental production of gastric and duodenal ulcers with caffeine stimulated our interest in this problem and prompted the present investigation.

METHODS

Caffeine-beeswax mixture. The method of embedding a chemical compound in a beeswax-mineral oil mixture was devised by Code and Varco (6) to study the prolonged, continuous plateau action of histamine. This mixture remains a semisolid mass when injected intramuscularly and has been shown to permit a slow and gradual absorption of the embedded histamine. Code, Varco, Wangensteen and coworkers (7, 8) experimentally produced gastric and duodenal ulcers in various laboratory animals by the intramuscular implantation of histamine in beeswax. In an attempt to obtain prolonged and continuous caffeine action, the technique of embedding caffeine in a beeswax-mineral oil mixture was used in this study.

The caffeine mixture was prepared in batches consisting of 22.5 grams of anhydrous caffeine alkaloid (Mallinckrodt), 15 cc. of melted bleached beeswax, and 60 cc. of hot mineral oil. The caffeine is kept dry before mixing with the beeswax by desiccation over sulfuric acid. If this precaution is not taken, a

lumpy granular mixture results which makes injection difficult. The caffeine is ground to a smooth homogeneous powder in an ordinary porcelain mortar. The weighed caffeine powder is placed in a hot mortar (over a fitted steam bath) and 15 cc. of melted beeswax is added with stirring. When a smooth paste is obtained, 60 cc. of hot mineral oil is added dropwise, continuously mixing with the pestle until the contents are smooth and homogeneous. The molten caffeine-beeswax-mineral oil mixture is allowed to cool to 40°C., drawn into warmed tuberculin syringes, and injected intramuscularly through 20 gauge needles. This mixture gives approximately 300 mg. of caffeine base per cubic centimeter.

Technique of administration. The caffeine mixture was injected alternately into the shoulder, thigh, calf and back muscles of a series of 22 cats. The injections were made daily, as a rule, at 6 P.M. A control series of 10 cats received an equal quantity of a warmed mixture of mineral oil and beeswax in the same proportions as used in the caffeine preparation. This vehicle has been shown to have no effect upon the gastroduodenal mucosa (7). The experimental animals were sacrificed when they showed persistent, marked anorexia for 3–5 days, weight loss, tarry stools, convulsions or evidence of complicating pneumonic involvement.

The diet consisted of milk and ground beef heart on alternate days. The cats were fed in the morning and the food pans removed from the cages at the time of injection in the evening. Because of the anorexia which developed in the experimental cats that later proved to have erosions or ulcers, it was decided to starve three of the control cats. All of the animals were allowed free access to water.

Acute experiments. In an attempt to explain the pathogenesis of the acute and subacute caffeine ulcers, studies were made to determine the effect of caffeine on gastric secretion. The results of the experiments will be published later, along with the effect of caffeine on gastric secretion in man. Acute experiments on cats were conducted using a modification of the technique devised by Lim (9). The cat was given no food on the day before the experiment, but allowed free access to water. Light chloroform anesthesia is used with a tracheal cannula. A ligature is placed about the cardio-esophageal junction, excluding the vagi by previous dissection, to avoid swallowed mucus and saliva. A loose ligature is placed about the pyloroduodenal junction, avoiding the neighboring vessels. A third ligature is tied tightly one-half inch below on the duodenum, again avoiding the vessels. The duodenum is opened between the second and third ligatures and a perforated rubber cannula is inserted through the pylorus into the stomach. The perforated rubber cannula is connected to a rubber tube of smaller diameter by a short piece of glass tubing over which

the pyloric ligature is tied securely. The rubber tube is brought to the exterior through a small stab wound in the right abdominal wall opposite the pylorus. The stomach is rinsed several times with physiological saline solution and the contents of the stomach are continuously drained through the cannula and rubber tube. With cats prepared in this manner, the effect of caffeine on gastric secretion was studied. The response to caffeine was compared alternately to that of histamine. Caffeine was administered intramuscularly or intravenously in doses of 125 to 250 mg. of caffeine as an aqueous solution of caffeine with sodium benzoate. The histamine control was given subcutaneously in doses of 0.1 to 0.6 mg. as the dihydrochloride. Between injections of the stimulating agents, sufficient time was allowed for the gastric secretory response to return to the basal level. The duration of the acute experiments varied from 5 to 9 hours.

RESULTS

Experimental production of acute and subacute ulcers in cats. Twenty-two adult cats, weighing from 3.5 to 7.8 pounds, were given 300 milligrams of caffeine base daily. Eleven of these (50 per cent) developed bleeding erosions or ulcers (Table I). All of the mucosal defects appeared in the lesser curvature of the stomach, in the antrum and prepyloric regions. The stomachs of 5 cats showed multiple lesions. One of the cats (No. 25) was pregnant at the start of the experiment but aborted the premature fetuses after five injections.

The stomachs contained black-brown stained material and showed numerous prominent bleeding points surrounding the ulcers. Grossly the mucosal defects resembled those occurring spontaneously in man. The edges of the subacute ulcers were sharply demarcated, with elevation of the surrounding moderately indurated mucosa (Figs. 2, 3, 4). The size of the ulcer varied from 2 to 8 mm. in diameter. The base of the larger ones was covered with a gray exudate, but in most instances there was old blood in the floor of the crater and fresh bleeding points in the surrounding tissues. In several of the cats, the duodenum contained a reddish-brown fluid.

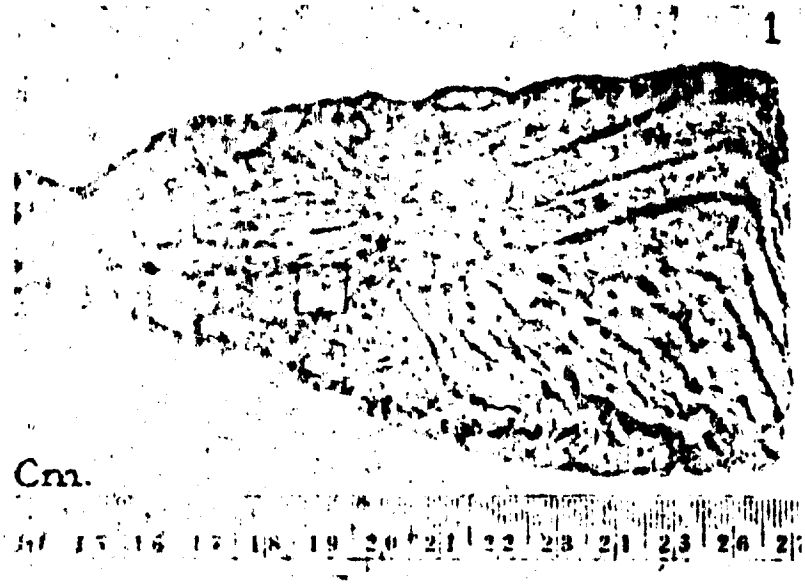
The acute and subacute, bleeding erosions and ulcers observed in the gross specimens were confirmed and studied in detail in the microscopic sections. Where the ulcerating process had not progressed too far, cells of the glandular epithelium showed karyolysis and pyknosis of the nuclei and fragmentation and granularity of the cytoplasm. The following summary (Cat No. 5—Fig. 8) illustrates the typical microscopic findings in this series of ulcers:

The structure and cellular detail is well preserved except for a broad, shallow ulcer. The floor and walls of the ulcer are covered by a thin necrotic membrane. In the undermined portion at the edge of the ulcer can be seen a

TABLE I

Production of ulcer in cats with caffeine-beeswax mixture

CAT NO.	SIZE	DOSE OF CAFFEINE	NO. OF INJECTIONS	NO. OF DAYS	RESULTS	REMARKS
	lbs.	mgs.				
1	4	300	6	6	Prepyloric bleeding ulcer	Sacrificed—convulsions
2	5	300	10	12	Two hemorrhagic areas in antrum	Expired—bronchopneumonia
3	4.8	300	27	33	No hemorrhage, erosion or ulcer	Sacrificed
4	5.3	300	6	7	Two bleeding ulcers in antrum	Expired
5	4	300	6	7	One bleeding ulcer in antrum	Sacrificed—convulsions
6	3.5	300	10	11	Two bleeding ulcers in antrum	Sacrificed
15	4.8	300	8	10	One bleeding prepyloric ulcer	Expired—antral erosion, duodenal hemorrhage
17	5.5	300	17	23	No hemorrhage, erosion or ulcer	Sacrificed
18	5.5	300	6	7	Antral erosion, duodenal hemorrhage	Expired—bronchopneumonia
19	5	300	16	22	No hemorrhage, erosion or ulcer	Sacrificed
21	4.5	300	2	3	Multiple bleeding erosions and ulcers	Expired
22	4.5	300	5	5	Shallow bleeding prepyloric ulcer	Expired—convulsions
25	4.3	300	30	38	One ulcer, two erosions in antrum	Expired—pregnant at start of experiment
30	6.8	300	11	16	No hemorrhage, erosion or ulcer	Expired—bronchopneumonia
31	5.5	300	3	4	Prepyloric ulcer	Expired
33	4.8	300	11	15	Prepyloric submucosal hemorrhage	Expired
35	5.5	300	30	38	No hemorrhage, erosion or ulcer	Expired
36	5.0	300	30	39	No hemorrhage, erosion or ulcer	Sacrificed
37	5.3	300	11	15	No hemorrhage, erosion or ulcer	Expired—bronchopneumonia
38	7.8	300	11	16	No hemorrhage, erosion or ulcer	Expired—convulsions
39	5	300	30	37	No hemorrhage, erosion or ulcer	Sacrificed
40	3.5	300	8	11	Bleeding prepyloric ulcer, three erosions	Expired
Ave.	5.0	2.5 gms.	8.4	10.2	11 of 22 cats developed erosions or ulcers	



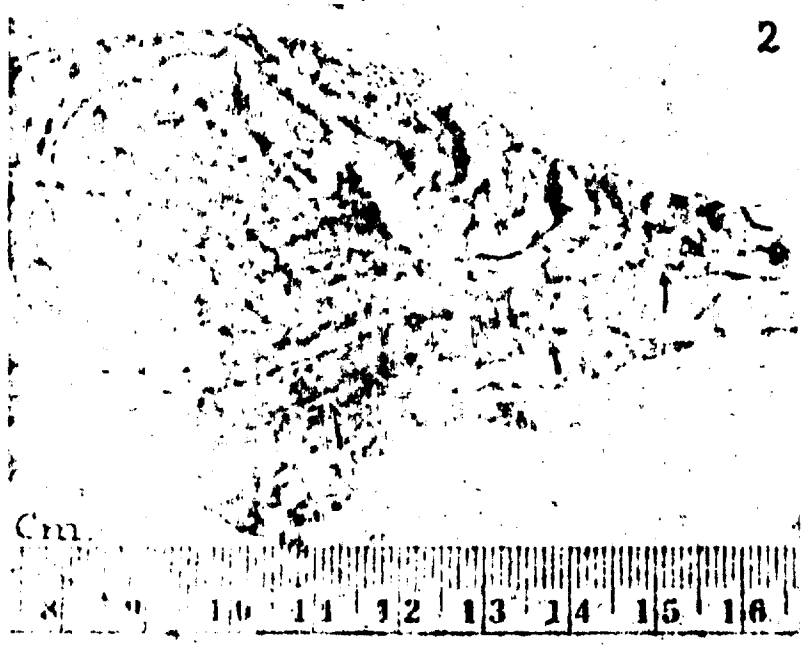
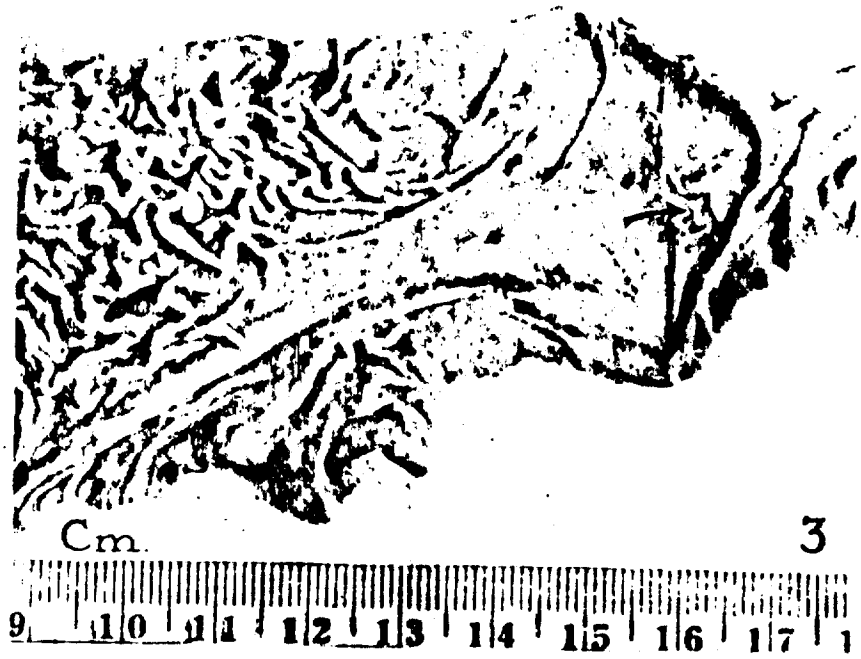


FIG. 1. Multiple diffuse areas of superficial desquamation of the epithelium of the gastric mucosa in cat (#52). Produced in the course of a study on gastric secretion in an acute experiment of 5 hours duration. Doses: 125 mg. caffeine (as the sodium benzoate), intramuscularly; 250 mg. caffeine, intramuscularly; 0.6 mg. histamine di-hydrochloride, subcutaneously.

FIG. 2. Multiple bleeding erosions and ulcers in stomach of cat (#21) receiving daily intramuscular implantations of 300 mg. caffeine (base) in mineral oil-beeswax [illegible] 3 days.



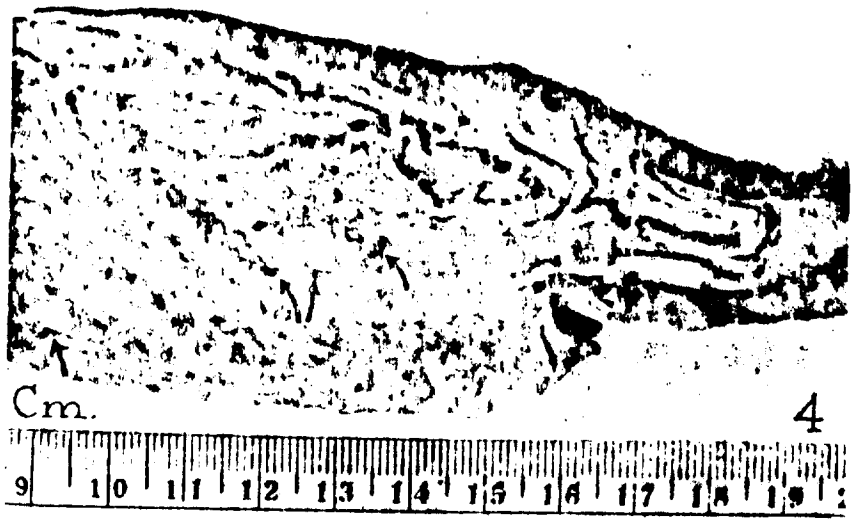


FIG. 3. Large bleeding ulcer in antrum, surrounded by bleeding erosions. Cat (#5) received daily intramuscular implantations of 300 mg. caffeine (base) in mineral oil-beeswax mixture. Sacrificed (convulsions) after 7 days.

FIG. 4. Large prepyloric ulcer in cat (#40), receiving daily intramuscular implantations of 300 mg. caffeine (base) in mineral oil-beeswax mixture. Expired after 11 days.

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FIG. 5. Photomicrograph showing erosion of gastric mucosa and desquamation of the glandular epithelium (Cat. #52). Swelling of collagenous fibers and edema of interstitial tissues of mucosa and submucosa. (×55)

FIG. 6. Photomicrograph of caffeine ulcer (Cat. #21, Fig. 2). A thrombosed vessel lies in the base of the ulcer bed. (×55).

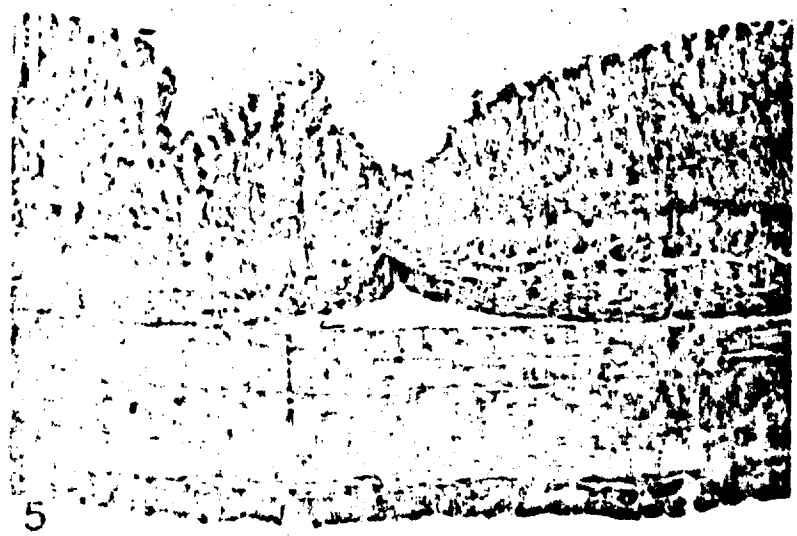
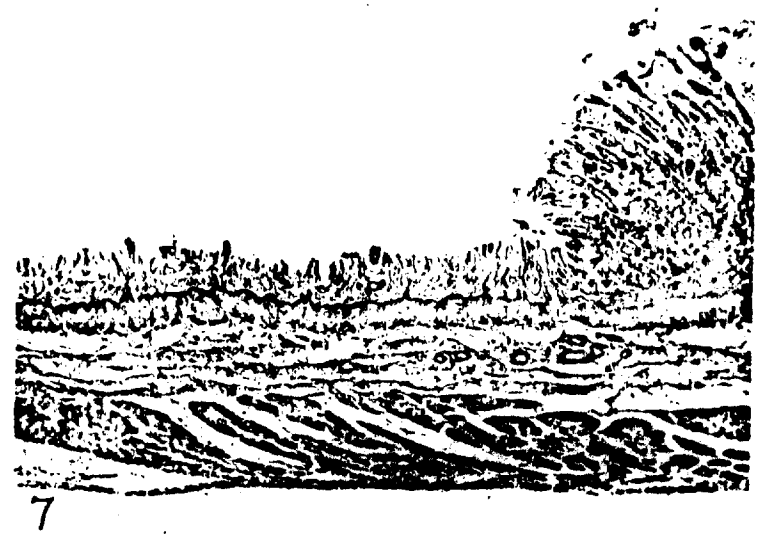


FIG. 5. Photomicrograph showing erosion of gastric mucosa and desquamation of the glandular epithelium (Cat. #52). Swelling of collagenous fibers and edema of interstitial tissues of mucosa and of muscularis. (×55).

FIG. 6. Photomicrograph of caffeine ulcer (Cat. #21, Fig. 2). A thrombosed vessel lies in the base of the ulcer bed. (×55).



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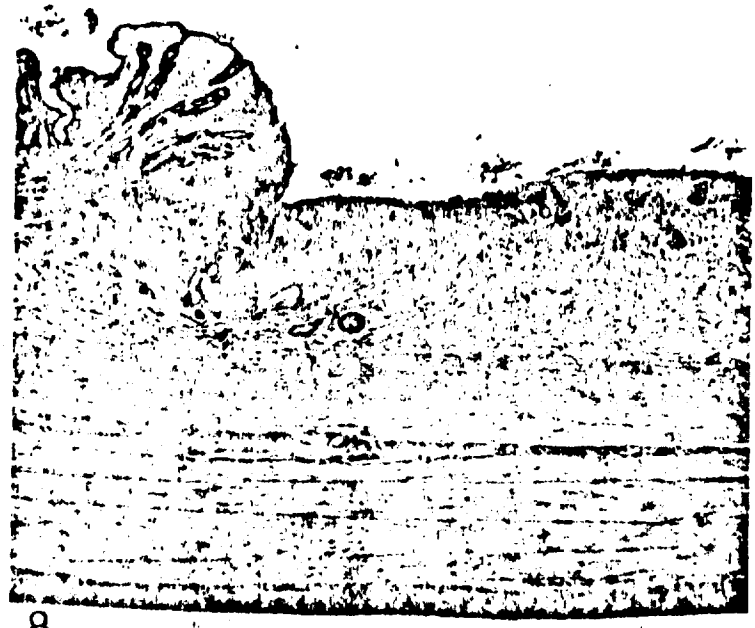

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FIG. 7. Photomicrograph of broad shallow ulcer in cat (#40, Fig. 4). (×55).

FIG. 8. Photomicrograph of caffeine ulcer (Cat #5, Fig. 3) located in prepyloric antrum. Note the undermining process, thrombosed vessel and extravasation of blood elements at the edge, collections of serous transudate, vascular dilatation and cellular infiltration of fibrinous ulcer bed. (×55).

thrombosed vessel, extravasated blood and a large amount of fibrin deposit. The muscularis mucosa is destroyed, and the submucosa involved in the ulcerative process. The latter showed serous transudate, lymphocytic and polymorphonuclear infiltration and moderate hyperemia. The muscularis and serosa show no evidence of change.

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FIG. 9. Photomicrograph of caffeine ulcer in cat (#6), showing rolled undermining edges and slough with marked cellular infiltration. (×55.)

The stomachs of the 10 *control cats* showed no evidence of hemorrhage, erosion or ulceration.

Acute experiments. After studies were made on the effect of caffeine upon gastric secretion, the cats were sacrificed and the gastric mucosa examined. Most of the stomachs contained a brownish red colored mucus and diffuse bleeding erosions. The surface of the mucosa appeared distinctly white and slightly elevated in *large diffuse areas* in contrast to the apparently uninvolved mucosa (Fig. 1). These gross lesions were not obtained in a control group of

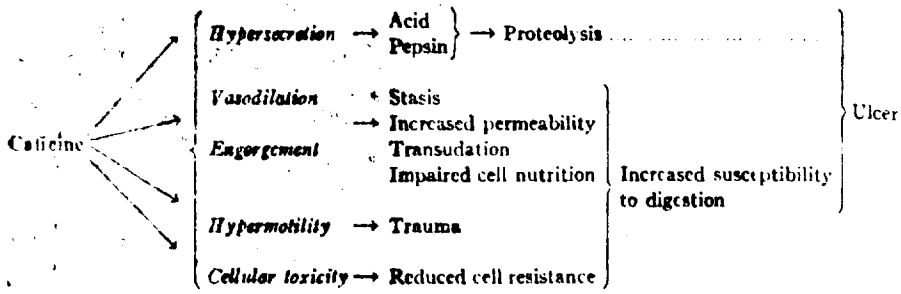
cats when the gastric secretion was stimulated continuously by histamine alone for as long as 10 to 12 hours. However, when a single injection of caffeine (125 mg., intravenously) was superimposed upon the continuous histamine stimulation, these large diffuse areas of superficial erosion resulted.

Microscopically (Fig. 5) these areas show superficial desquamation and disintegration of the glandular and surface epithelium. The arterioles, venules and capillaries of the mucosa and submucosa appear markedly dilated and engorged with blood. The submucosa is thickened in places and its collagen strands widely separated and swollen. Collections of serous transudate appear in the mucosa and submucosa.

DISCUSSION

Hanke (1) produced gross and microscopic erosions of the gastric mucosa in cats by the subcutaneous injection of toxic doses of an aqueous solution of caffeine sodium salicylate. The stomachs contained acid contents which Hanke attributed to the caffeine. He offered these results as evidence of acid gastric juice producing peptic lesions. Hanke (2) also succeeded in producing chronic gastric ulcers by the daily subcutaneous injection of 200 to 300 mgs. of caffeine sodium salicylate. Grasso (3) failed to produce any gastric lesions in rabbits with an infusion of coffee administered orally over a month, but obtained an ulcerative gastritis in rabbits when he gave caffeine plus nicotine parenterally. No definite conclusion regarding the role played by caffeine can be drawn from an experiment of this type. Molteni (4) produced erosive and ulcerative lesions in the antrum and pylorus of 40 per cent of rats by the daily subcutaneous injection of 20 to 30 mgs. of caffeine sodium salicylate over a period of time varying from 10 to 70 days. In another series of rats, injected with caffeine daily for 60 days, the dose was stopped and the animals sacrificed at 8, 15, and 20 days. In this group no ulcerations were found and Molteni concluded that the lesions *heal rapidly*. He also found more acid and a larger volume of gastric contents in the stomachs of test animals than in the controls. This he concluded was evidence that a hypersecretion of an acid gastric juice was an important factor in the pathogenesis of ulcers by caffeine. Judd (5) used a caffeine-beeswax-mineral oil mixture, similar to the one used in our experiments, and obtained ulcers in 40 per cent of guinea pigs and cats. The guinea pigs were injected daily with 100 mg. of caffeine, intramuscularly, and developed ulcers in an average of 4.5 days. The cats received 300 mg. of caffeine daily and developed ulcers in an average time of 14.5 days. The ulcers were for the most part gastric in location. There was one associated duodenal ulcer and one duodenal ulcer without a gastric lesion. He too suspected the mechanism was the constant elaboration of an excessively acid gastric juice.

The problem of the pathogenesis of these ulcers produced by caffeine is under investigation at present, but the factors which *may* play a role can be diagrammatically enumerated as follows:



There is no uniformity of opinion among the earlier investigators as to the effect of caffeine and its beverages upon gastric secretion. However we have considerable evidence (10) to show that caffeine and coffee when taken orally are potent stimulants of both the acid and pepsin content of gastric juice in man and cat, but not the dog in the same doses. The intramuscular or intravenous administration of caffeine likewise provokes a copious flow of gastric secretion. Caffeine does not seem to give much stimulation to the secretion of mucus. Dragstedt (11) concludes that the excessive continuous secretion of gastric juice with relative or absolute deficient neutralization is one of the major factors in the pathogenesis of human gastro-duodenal ulcers. It seems highly probable that the ulcers produced in this study developed *in part* as a consequence of the presence in the stomach, when it was empty of food, of a strongly acid gastric juice over a long period of time. There may or may not be a neurogenic factor by virtue of the excitatory action of caffeine upon the central nervous system.

The microscopic picture suggests that the marked vasodilation and engorgement might in turn give rise to stasis, increased capillary permeability, transudation, and impaired cell nutrition; all of which would contribute to the development of ulceration. Direct observation of a mucosal stoma in the cat shows a persistent blushing (hyperemia) after the intravenous administration of caffeine, followed by cyanosis. Preliminary experiments indicate that caffeine does not induce pylorospasm, but rather increases peristaltic contractions to accelerate gastric emptying. It has been suggested that the trauma from vigorous contractions might initiate a mucosal defect when the latter is engorged and edematous. In their experiments upon a modern "Alexis St. Martin," Wolf and Wolff (12) observed that "intense anxiety, hostility and resentment were accompanied by severe and prolonged engorgement, hypermotility and hypersecretion in the stomach. In this state mucosal erosions

and hemorrhages were readily induced by even the most trifling traumas and frequently bleeding points appeared spontaneously as a result of vigorous contractions of the stomach wall." Inasmuch as the acute experiments showed a diffuse desquamation of the mucosal epithelium in large areas, this suggests that caffeine may provide a factor of "cellular toxicity" in the doses used and hence lower the resistance of the cells to the ulcerative process. The concensus today is that "ulcer of the stomach is in some way due to a local loss of resistance on the part of the mucous membrane to the digestant activity of the gastric juice" (11). Whether or not any or all of the factors discussed above play a role in the pathogenesis of caffeine ulcers produced experimentally in cats is purely speculative and the subject is still under investigation.

SUMMARY AND CONCLUSIONS

The prolonged continuous intramuscular administration of caffeine in a mixture of beeswax and mineral oil is capable of producing gastric ulcers in cats. That the *excessive use* of caffeine-containing beverages might play a *contributory* role in the pathogenesis of gastro-duodenal ulcers in man is implied. A speculative discussion of some of the most probable factors involved in the development of experimental gastric ulcers in cats is presented. In view of these findings and the marked stimulation of gastric secretion in man by the oral or parenteral administration of caffeine, it is suggested that the use of caffeine beverages be restricted in the management of the peptic ulcer patient.

REFERENCES

1. Hanke, H.: Arch. f. Klin. Chir., **178**: 607, 1934.
2. Hanke, H.: Klin. Wochenschrift, **13**: 978, 1934.
3. Grasso, R.: Zit. nach Rona, **96**: 160, 1936.
4. Molteni, M.: Arch. Ital. di Chir., **53**: 135, 1938.
5. Judd, E. S.: Bull. Am. Coll. of Surg., **28**: 46, 1943.
6. Code, C. F., and Varco, R. L.: Proc. Soc. Exper. Biol. & Med., **44**: 475, 1940.
7. Walpole, S. H., Varco, R. L., Code, C. F., and Wangensteen, O. H.: Proc. Soc. Exper. Biol. Med., **44**: 619, 1940.
8. Hay, L. J., Varco, R. L., Code, C. F., and Wangensteen, O. H.: Surg. Gyn. & Obst., **75**: 170, 1942.
9. Lim, R. K. S.: Quart. J. Exper. Physiol., **13**: 71, 1923.
10. Roth, J. A., and Ivy, A. C.: Unpublished data.
11. Dragstedt, L.: Arch. Surg., **44**: 438, 1942.
12. Wolf, S., and Wolff, H.: J. A. M. A., **120**: 670, 1942.

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THE EFFECT OF CAFFEINE UPON GASTRIC SECRETION IN THE DOG, CAT AND MAN

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We have recently confirmed the work of Judd (1) on the experimental production of acute and subacute gastric ulcers in cats by the intramuscular implantation of caffeine in a beeswax-mineral oil mixture (2). It is to be suspected that the constant elaboration of a highly acid gastric juice might be one of the factors involved in the pathogenesis of such ulcers. However, there is no uniformity of opinion among investigators as to whether caffeine does or does not stimulate gastric secretion.

Caffeine has been shown consistently to have no effect upon gastric secretion in Pavlov and Heidenhain pouch dogs (1, 3, 4, 5, 6, 7, 8). Actual investigation of the influence of caffeine upon gastric secretion in the cat has not been made, but an excito-secretory effect has been suspected (1, 9, 10). Considerable disagreement is found in the literature concerning the effect of caffeine in man. Various authors (6, 11, 12, 13, 14) conclude that caffeine does not stimulate gastric secretion in man; whereas others (15, 16, 17, 18) have observed an increased output of acid gastric juice after caffeine administered orally or parenterally. The experimental design and technique used in some of the investigations on human subjects was poor in the light of current knowledge.

The purpose of the present investigation was to determine whether caffeine stimulates gastric secretion in man, cat and the dog, and whether the apparent species difference does exist.

METHODS. *Dogs.* Four dogs with Pavlov pouches of the stomach and two dogs with gastrostomies were used in these studies. In all experiments, before caffeine was administered, the animals were allowed to become basal, i.e., secreting no free acid. The dose of caffeine used was 250 mgm. as the sodium benzoate. The caffeine was usually given subcutaneously, but in a few experiments the intramuscular and intravenous routes were used. Following the injection of caffeine, the gastric juice was collected for a two-hour period. In order to demonstrate that these gastric pouches were capable of responding to a known stimulus, histamine was injected subcutaneously two hours after the administration of caffeine and the gastric juice collected for another one-hour period. The histamine used was the dihydrochloride; the dose varied from 0.2 to 0.4 mgm., but was kept constant for any given animal.

Cats. Acute experiments on cats under chloroform anesthesia were conducted using a modification of the technique devised by Lim (19) which consists of ligation of the cardio-esophageal junction, excluding the vagi by previous dissection, and cannulation of the stomach with a perforated rubber tube inserted through the pylorus. With cats prepared in this manner, the effect of caffeine on gastric

secretion was studied. Caffeine was administered intravenously in doses of 65 to 125 mgm. as an aqueous solution of caffeine with sodium benzoate. As the drops of gastric juice fell from the cannula, they were recorded on a slowly moving kymograph. From this record the rate of flow (drops per minute), latent period and duration of response were calculated.

In another group of experiments, 100 mgm. of caffeine in 20 cc. of distilled water, neutralized to pH 6, was introduced through the pyloric cannula and allowed to remain in contact with the gastric mucosa for 30 minutes. After this interval the gastric contents were drained and the stomach rinsed twice with distilled water. The gastric secretion was collected for a period of 90 to 120 minutes, washing the stomach every 30 minutes.

Human subjects. Fractional gastric analyses were conducted on human subjects using a "block" technique to permit exposure of the gastric mucosa to the test meal for a period of 30 minutes. All tests were performed after a fast of 10 to 12 hours. To obtain the "basal" level of secretion, the stomach was emptied completely of its contents every 10 minutes for a control period of one-half hour. By means of a large syringe, 200 cc. water (temp. 37–40°C.) containing 250 mgm. sodium benzoate was introduced into the stomach via the Rehfuss tube, serving as a control for the volume and chemical influence of sodium benzoate in the caffeine test meal. For the next two 10-minute intervals, 10 cc. samples of gastric contents were removed for analysis. The purpose in removing these samples was to detect the first rise in acid concentration and thus the latent period. Thirty minutes after the control test meal was introduced and every ten minutes thereafter until the secretory response had subsided and the "basal" level once again was obtained, the stomach was emptied completely of its contents. As pointed out by Bloomfield and Keefer (20) the complete emptying of the stomach is made more certain by having the subject lean backward, forward, and from side to side as the stomach is aspirated. Having thus obtained the "basal" level of secretion and the secretory response to a control test meal, an equal volume of water containing 250 mgm. caffeine as the sodium benzoate was introduced via the stomach tube and the "block" procedure again conducted as outlined above.

The total output of acid was calculated on the basis of the titration values for the two 10 cc. samples plus the complete recovery every 10 minutes thereafter for a period of 1 hour. Thus, since the volume of gastric contents aspirated depends upon not only the secretion but the amount lost through the pylorus, the total output of acid would be greater than that represented in the data.

Another group of experiments were made on human subjects to study the gastric secretory effect of caffeine administered intramuscularly. After a "basal" control period of 30 minutes, 250 mgm. caffeine were injected intramuscularly and the total gastric contents aspirated every 10 minutes for at least one and one-half hours.

All samples of gastric juice in these experiments were titrated against 0.0278 N NaOH (1 cc. equals 1 mgm. HCl) for free and total acidity, using Töpfer's reagent (pH 3.5) and phenolphthalein (pH 8.5) respectively. In all cases the responses are expressed as the output of acid in milligrams of HCl.

RESULTS. *Dogs.* The results of 32 experiments on 6 dogs are summarized in the accompanying table 1. In no instance did the parenteral administration of caffeine stimulate gastric secretion in the dog, but histamine gave rise to its usual

TABLE 1

The gastric secretory response to caffeine and histamine in dogs

DOGS	DOG NO.	NO. OF EXPTS.	AVE. POST-CAFFEINE RESPONSE (MGM. HCl/2 HRS.)		AVE. POST-HISTAMINE RESPONSE (MGM. HCl/1 HR.)	
			Free acid	Total acid	Free acid	Total acid
Pavlov pouch dogs	P-1	8	0.0	2.7	57	75
	P-2	6	0.0	7.8	53	66
	P-3	6	0.0	5.2	76	97
	P-4	6	0.0	2.3	31	46
Gastrostomy dogs	G-1	3	0.0	5.0	23	32
	G-2	3	0.0	11.0	54	98
Ave.	6	32	0.0	5.8	49	69

TABLE 2

The gastric secretory response to intravenous caffeine in cats*

CAT NO. VAGI INTACT	PRE-CAFFEINE RATE	LATENT PERIOD	MAX. POST-CAFFEINE RATE	DURATION OF RESPONSE	VOLUME AND MGM. FREE HYDROCHLORIC ACID			
					Pre-caffeine (10 min.)		Post-caffeine (60 min.)	
					c.c.	mgm. HCl	c.c.	mgm. HCl
	drops/min.	*min.*	*drops/min.*	*min.*				
1	0.33	20	5.00	30	0.4	0.0	8.3	41.8
2	0.08	13	1.84	76	0.6	0.4	4.5	6.6
3	0.38	13	1.23	34	0.3	0.2	2.7	11.6
4	0.64	20	1.17	48	0.2	0.6	2.9	6.7
5	0.34	12	1.03	39	0.1	0.0	2.7	11.0
6	0.23	7	4.00	50	0.3	0.0	8.3	20.6
7	0.11	12	1.89	30	0.2	0.1	2.2	2.0
8	0.12	10	2.58	61	0.1	0.0	5.2	14.2
9	0.41	10	1.36	39	0.2	0.0	5.2	22.1
10	0.23	6	3.86	47	0.1	0.1	5.9	19.4
11	0.38	9	1.50	35	0.2	0.0	4.1	8.8
12	0.06	12	3.00	56	0.3	0.0	7.4	29.4
13	0.40	17	2.60	40	0.1	0.0	2.3	13.5
14	0.20	10	2.50	50	0.2	0.0	3.3	15.7
Ave. . .	0.26	12	2.4	45	0.23	0.1	4.7	16.0

* Dose: 125 mgm. caffeine as the sodium benzoate. Cat 13 received 65 mgm.

excitosecretory response. Lavage of the stomach with caffeine in Pavlov and total pouch dogs does not provoke a stimulation of gastric secretion.

Cat. The data in table 2 show that the intravenous injection of caffeine in anesthetized cats provokes a marked stimulation of gastric secretion. The usual

sequence of events following the intravenous administration of caffeine include: marked mydriasis for 15 to 30 minutes, hyperpnea, and, after an average latent period of 12 minutes, the increased flow of acid gastric juice was manifest, lasting on the average for a period of 45 minutes.

TABLE 3

Average gastric secretory response to lavage with caffeine in cats

	TIME	VOLUME	FREE ACID	TOTAL ACID
	minutes	*cc.*	*mgm. HCl*	*mgm. HCl*
Control secretion	30	0.4	0.0	1.4
Caffeine soln. applied	30	20.0	0.0	6.0
Caffeine soln. returned		20.7	1.8	7.3
Post-application secretion	30	1.8	6.2	7.6
Post-application secretion	30	1.0	2.7	3.6
Post-application secretion	30	0.7	1.1	1.9

TABLE 4

The gastric secretory response to oral administration of caffeine in human subjects

SUBJECT	CONTROL: 200 CC. WATER + 250 MGM. SODIUM BENZOATE			EXPERIMENTAL TEST: 200 CC. WATER + 250 MGM. CAFFEINE—ORAL					PER CENT OF STIMULATION (FREE ACID)[1]
	Total juice secreted in 1 hour:			Latent period	Duration of response	Total juice secreted in 1 hour:			
	Volume	Free acid	Total acid			Volume	Free acid	Total acid	
	cc.	*mgm. HCl*	*mgm. HCl*	*minutes*	*minutes*	*cc.*	*mgm. HCl*	*mgm. HCl*	
J. R.	51	15	27	15	30	73	98	113	550
J. R.	51	7	16	15	50	55	55	63	685
E. P.	67	99	115	15	40	95	195	218	97
J. I.	180	228	282	15	60+	199	363	435	59
J. W.	42	12	23	15	50	48	36	48	200
J. A.	139	9	33	15	50	77	28	49	211
P. H.	46	17	29	15	60	94	181	218	964
J. J.	58	67	81	15	60+	142	337	392	403
F. J.	81	190	212	15	60+	123	393	441	107
G. H.	64	60	82	25	60	93	152	186	153
M. S.	52	47	64	25	60	61	107	144	128
Ave.	75	69	88	17	53	93	177	210	324

In 15 experiments on 9 cats, lavage of the stomach for 30 minutes with caffeine resulted in a stimulation of gastric secretion as measured by the volume, free acid and total acid response (table 3). The same volume of water (20 cc.) has been shown to have no effect in a series of control experiments.

Man. In table 4 are presented the individual variations in the gastric secretory response to the administration of caffeine via the stomach tube in human subjects.

In general three types of secretory curves are obtained by the fractional gastric analysis with a caffeine test meal. Approximately 85 per cent of human subjects give an abrupt curve which returns to its basal level within 50 to 70 minutes; 10 per cent describe a low flat curve (subjects J. W. and J. A.) which subsides within 60 to 90 minutes; and about 5 per cent show a prolonged secretory response at a high plateau level (subjects J. I., J. J. and F. J.). In a comparison between the response to the control (200 cc. water with 250 mgm. sodium benzoate) and to the caffeine test meals (table 4) it appears that the average volume of secretion is not much greater after caffeine (93 cc. compared to 75 cc.). However, in using a "block" technique it must be remembered that only a sample is withdrawn at the first two intervals and then the stomach is emptied completely of its contents at the third interval. This results in the recovery of a relatively large volume at this interval in both test meals, although the acid value is but slightly elevated above the basal level in the control studies. The data on the caffeine test meal show that the stimulation of parietal cell secretion is manifest after a latent period of 15 to 25 minutes; i.e., the free acid concentration began to rise during the period between the 10 and 20 minute interval aspirations. The average free acid response is over two and one-half times greater for the caffeine test meal than that for the control in the same period of time; actually the total output is even greater because the response from caffeine is much more prolonged in all individuals than the response to the control tests.

In figure 1 the graph represents the composite gastric secretory response to a caffeine test meal in comparison with the effect of a control test meal in 10 human subjects. In analyzing the curves for acidity, the initial drop after the control test solution and to a less extent after the caffeine test meal is attributed to dilution by the test solutions inasmuch as only a sample of gastric contents is aspirated at these intervals. The abrupt rise then represents the recovery of gastric contents accumulating over the 30 minute period in which the mucosa is exposed to the stimulating agent. In this composite response the actual peak of secretion resulting from the caffeine stimulation occurs 40 to 50 minutes after the test solution was introduced. In the block form of graphic representation can be seen the average maximum secretory response in volume, free acid and total acid per 10 minutes in comparison with the average pre-caffeine control levels for a 10 minute period.

The results of the gastric secretory response to caffeine given intramuscularly in human subjects are summarized in table 5. This route of administration was used in man to demonstrate that caffeine stimulation is *not solely* dependent on the local effect of the drug on the mucosa. The tabulated data show the individual variations in latent period, duration of stimulation and the total output of gastric juice for a period of 90 minutes after the injection of caffeine. The same subjects who gave a prolonged response to the caffeine test meal via the stomach tube gave a similar response to the intramuscular caffeine but at a somewhat lower plateau level.

In figure 2 the composite secretory response to the intramuscular administration of caffeine in 11 human subjects is presented in the form of a graph. The

composite curves are misleading in so far as the latent period is concerned. The majority of subjects do not show evidence of stimulation for 50 to 60 minutes after the injection, but a few do after 20 to 30 minutes. Hence the early rise in the composite curves. The block graph shows the average maximum secretory response per 10 minutes after intramuscular caffeine in comparison with the average basal levels for the 10 minute interval preceding injection.

Discussion. The dog has been the most frequent choice of experimental animal for studies on the mechanisms of gastric secretion, on the production of

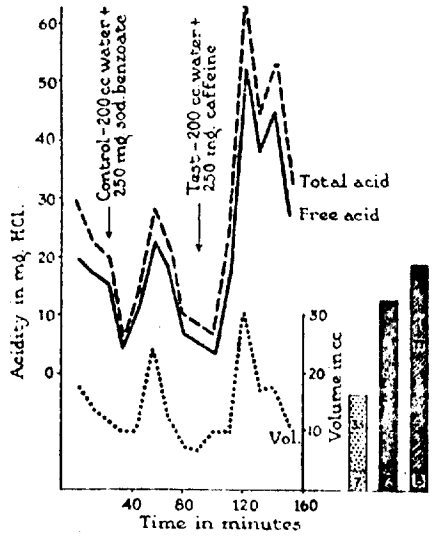

Fig. 1

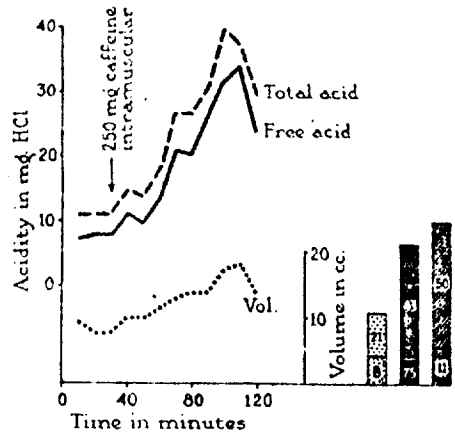

Fig. 2

Fig. 1. Composite curves showing the average total gastric secretory response of 10 human subjects to a control test meal (200 cc. water with 250 mgm. sodium benzoate) and a caffeine test meal (200 cc. water with 250 mgm. caffeine as a soluble sodium benzoate). Both free and total acid are expressed as milligrams of HCl. The block graph shows a comparison between the average pre-caffeine levels of volume, free acid and total acid per 10 minutes and the average maximum secretory response for 10 minutes.

Fig. 2 Composite curves showing the average total gastric secretory response of 10 human subjects to the intramuscular administration of 250 mgm. caffeine as the sodium benzoate. The block graph shows a comparison between the average pre-caffeine levels of volume, free acid and total acid per 10 minutes and the average maximum secretory response for 10 minutes.

gastroduodenal ulcers, and on the effectiveness of various therapeutic procedures on experimentally induced peptic ulcers. Therefore, the observation that caffeine does not stimulate gastric secretion in the dog but does in man and the cat is very significant and has far-reaching implications. That such a species difference does exist must constantly be borne in mind in applying observations made on the dog to various physiological phenomena in man. Do man and the cat possess a mechanism of gastric secretion absent in the dog? Or, is there an effective mechanism antagonistic to the caffeine excitosecretory effect in the dog, and absent in man and the cat?

That earlier investigators failed to show conclusively that caffeine has a definite excitosecretory effect upon the stomach of man can in part be attributed to faulty technique, such as insufficient exposure of the gastric mucosa to caffeine (oral) or failure to wait long enough for intramuscular caffeine to produce its effect. We have observed the peak of gastric secretion to occur 40 to 50 minutes after the ingestion of caffeine and 70 minutes after intramuscular administration. To further clarify the confusion in the literature regarding the effect of caffeine and caffeine-containing beverages upon gastric secretion, the following may be said: Stimulation of gastric secretion by caffeine and coffee (or de-caffeinated preparations) are not one and the same. Thus, although a so-called de-caffeinated coffee preparation was found to provoke a flow of acid gastric juice only slightly different from that obtained from crude or roasted coffee (13), this is not evidence that caffeine does not stimulate gastric secretion in man. Nor can one conclude that the roasting process does not produce a gastric secretagogue from the crude green coffee bean. Coffee made from roasted beans may stimulate gastric secretion due to the effect of one or all of the following: *1*, its caffeine content; *2*, a natural secretagogue present in the green coffee bean; *3*, a product of the roasting process, or *4*, irritant volatile oils.

TABLE 5

The gastric secretory response to the intramuscular administration of caffeine in human subjects

SUBJECT	CONTROL PERIOD: BASAL SECRETION				EXPERIMENTAL TEST: 250 MGM. CAFFEINE—INTRAMUSCULAR					
	Basal volume	Secretion for ½ hr.		Pre-injection conc.†	Latent period	Maximum post-injection conc.†	Duration of response	Total volume	Secretion for 1½ hrs.	
		Free acid	Total acid						Free acid	Total acid
	cc.	*mgm. HCl*	*mgm. HCl*	*mgm./cc.*	*min.*	*mgm./cc.*	*min.*	*cc.*	*mgm. HCl*	*mgm. HCl*
J. R.	10	2	6	0.16	60	2.15	80	83	105	137
J. R.	18	6	13	0.29	50	1.57	80	83	77	121
M. G.	21	22	30	0.94	40	3.72	70+	170	438	525
J. R.	25	11	21	0.00	30	1.86	70	136	171	224
J. I.	50	99	123	1.73	60	2.86	60+	194	438	525
E. P.	23	66	76	2.17	60	4.07	50	76	253	296
J. R.	24	2	10	0.33	60	1.30	50	104	62	101
J. W.	29	7	24	0.20	50	1.28	40	104	80	135
F. J.	14	45	50	3.03	50	3.33	70+	115	334	383
J. A.	37	2	19	0.00	50	0.80	40	77	20	51
J. J.	12	25	30	1.30	40	2.19	70+	181	307	396
G. H.	49	4	22	0.14	30	1.73	70	181	132	211
J. N.	30	20	35	0.32	30	2.85	30	106	147	199
R. N.*	14	0	15	0.00	20	1.34	40	143	173	216
Ave.	25	22	34	0.77	45	2.22	63	125	196	251

* Secretion aspirated for only 70 minutes in this case.

† Concentration of free acid as milligram HCl per cubic centimeter per 10 minute period.

Inasmuch as caffeine, administered parenterally or by direct lavage of the stomach, does stimulate gastric secretion in the cat, the constant elaboration of a highly acid gastric juice might be one of the factors involved in the experimental development of caffeine ulcers in cats. Preliminary experiments on human subjects have also shown that caffeine stimulates the output of pepsin as well as hydrochloric acid. Thus, the excessive use of caffeine-containing beverages may contribute to the pathogenesis of human peptic ulcer in the susceptible individual, or perpetuate an ulcer already existing. This is particularly suggested by the prolonged secretory response to caffeine obtained in a certain group of subjects who fall in the descriptive category of: "conscientious, hard-working individuals who in time of stress are outwardly composed but inwardly tense."

CONCLUSIONS

1. We have confirmed the observation of the earlier investigators that caffeine does not stimulate gastric secretion in the dog.

2. Caffeine, administered by the intravenous route or by lavage of the stomach, provokes a copious flow of acid gastric juice in the cat. The significance of this species difference is pointed out.

3. Caffeine, administered intramuscularly or by the oral route, stimulates gastric secretion in man.

REFERENCES

(1) Judd, E. S. Bull. Am. Col. of Surg. **28**: 46, 1943.
(2) Roth, J. A. and A. C. Ivy. Gastroenterology, in press.
(3) Pincussohn, L. Ztschr. f. physik. u. diat. Therap. **11**: 261, 1907. Münch. Med. Wchnschr. **53**: 1248, 1906.
(4) Bickel, A. Internat. Bietr. **1**: 365, 1910.
(5) Kestner, O. and B. Warburg. Klin. Wchnschr. **2**: 1791, 1923.
(6) Goldbloom, A. Arch. f. Verdgskrkh. **42**: 13, 1928.
(7) Petrowa, M. K. and S. Ryss. Arch. f. Verdgskrkh. **47**: 328, 1930.
(8) Wichels, P., J. Brink and H. Lauber. Ztschr. Klin. Med. **123**: 325, 1933.
(9) Hanke, H. Arch. f. Klin. Chir. **178**: 607, 1934. Klin. Wchnschrift. **13**: 978, 1934.
(10) Molteni, M. Arch. Ital. di Chir. **53**: 135, 1938.
(11) Miller, R. J., O. Bergeim, M. E. Rehfuss and P. B. Hawk. This Journal **52**: 28, 1920.
(12) Ehrmann, R. and L. Dinkin. Neue Dtsch. Klin. **6**: 574, 1930.
(13) Ohnell, H. and H. Berg. Acta. Med. Scandinav. **76**: 491, 1931.
(14) Eichler, O. Arch. f. Exper. Path. u. Pharmakol. **190**: 123, 1938.
(15) Katsch, G. and H. Kalk. Klin. Wchnschr. **4**: 2190, 1935.
(16) Holler, G. and J. Bloch. Arch. f. Verdgskrkh. **38**: 351, 1926.
(17) Cade, A. and M. Milhaud. J. de Med. de Lyon **10**: 523, 1929.
(18) Wichels, P. Ztschr. f. Klin. Med. **123**: 336, 1933.
(19) Lim, R. K. S. Quart. J. Exper. Physiol. **13**: 71, 1923.
(20) Bloomfield, A. L. and C. S. Keefer. J. A. M. A. **88**: 707, 1927.

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The Relationship between Caffein and Intermediary Metabolism

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Despite the continuous modifications produced by metabolic reactions, the chemical composition of living cells is maintained constant by delicate control mechanisms of enzyme activity capable of producing modifications in the whole enzyme sequence. The study of such an argument has grown in the last few years with a number of experimental data that allows us to consider a new aspect of cellular physiology, that of metabolic control.

The term was first coined to explain the characteristic increase of mitochondrial respiration produced by ADP (adenosin-dyphosphate) which determines an increased consumption of O_2 and of the oxidizable substrata, such as changing the mitochondrial respiration from its resting state, characterized by the term Stage IV, to a condition nearly ten times more active corresponding to Stage III. The control is specific and disappears when all the ADP is transformed, so that the phenomenon is noted in biochemistry with the name respiratory control. It helps explain other fundamental metabolic correlations, such as the inhibition of the glycolysis produced by O_2, discovered by Pasteur. More recently there is evidence of other types of control, among which is the so-called "allosteric" because it depends on the effect of particular metabolites that act on non-catalytic positions of the enzymes, and that in general accompany structural modifications of the enzymatic molecules.

The purpose of this work is to try to explain the action mechanism of a drug, such as caffeine, on the basis of a possible interaction with enzymes connected with the allosteric controls.

"Glucidic" metabolism

It is already known that the injection of caffeine in higher animals produces hyperglycemia, usually associated with the increase of the glycolysis and the consumption of O_2. In the isolated muscle the production of lactic acid is very elevated. All these results imply that the drug should act at the level of the enzymatic system responsible for the increase either of the glucose or the lactic acid, and namely at the phosphorylase level. Not enough was known about this very complex enzyme to define the question in its exact terms, except that an increased phsophorylase activity in the liver due to the presence of phosphatase provoked an increase in the production of glucose which spilled into the blood, whereas in the muscle where there was no phosphatase there was increased production of lactic acid, in which the anaerobic demolition of the glucose 1-phosphate was

increased.

Nature of the phospherylase

Today it is well known that the phsepherylase is one of the enzymes that besides the catalytic locations presents so-called regulatory locations because they are important in interacting with simple metabolic compounds capable of modifying the enzymatic speed in such a way as to vary the proceedings of an entire metabolic sequence. The enzyme extracted from the skeletal muscle by Cori, Cori, and Green in 1943 is a crystalline protein with a molecular weight of 380,000 equivalent to a tetramer made up of four monomers, each of them almost equal to each other.

Each monomer presents a "phosphoserine" necessary to the cztalytic activity and a molecule of pyridoxal phosphate bound to a residue of lysine. Only when the enzyme is found in such a structural conformation does it take the name phospherylase a to indicate that it is capable of converting the end chain of a glycogen molecule (with a transfering mechanism over a radical of inroganic phosphate) into a glucose 1-phosphate molecule and into an inferior glycogen residue of one unit.

Figure 1 demonstrates when an active form is attacked by an hydrolytic enzyme even that present in the muscle, a spodific phosphatase that hydrolyzes the four phospheric residues bound to the four residues of serin, the inactice enzyme. The inactivity corresponds to the break of the tetramer unit into two halves whose molecular weight is half of the original's, and is incapable of attacking the glycogen. To distinguish it from the first one, it takes the name of phospherylase b (inactive). The reaction is reversible but catalyzed by one other enzyme usually present in the muscle, the phospherylase b kinase which needs Ca, Mg, and ATP. It is clear that in such a way the concentration of active phospherylase in the cells can vary and consequently so does the importance of the glycogenolysis. The two forms of phospherylase a and b were found even in the liver, but their structure is partially different, as is the mechanism that determines activity.

The Working Process of Phospherylase

The first observations examined the effect of AMP on phospherylase b which in the presence of a nucleotide becomes active. Since then a considerable quantity of data has permitted Mened and colleagues to

first foresee then to demonstrate that phospherylase undergoes an allosteric process with consequent changes in conformation. It is still not possible to present a more precise model than that shown in Figure 1, because there are different factors in the process which complicate the mechanism of molecular attraction. Above all they modify the characteristic kinetic curve which expresses the allosteric process, the intervention of enzymatic macromolecules capable of producing structural modifications in other enzymes. It is a new physical dhemistry condition comparable to other rare examples of macromolecular interaction such as the process of synthetized glutamine, and in a certain sense the workings of the proteolytic enzymes connected with the coagulation of blood.

Faced with the numerous results obtained in this area with other fundamental results obtained in the physiological field, such as those relative to the effect of epinephrine and glucagon that cause hyperglycemia, a new field of research is opened whose scope is to correlate the connection of hormones and hyperglycemia with the mechanism action' action of the new enzymes in the workings of hepatic phospherylase. Such studies have identified a new metabolite, the so-called cyclic AMP with a chemical intermediary of hormone effects and also nervous impulses.

The cyclic AMP is a particular form of "diesterea" (precisely 3' - 5' phosphate) of a well known nucleotide adenosine 5-phosphate diffused in all the cells, which works off an enzyme discovered in the muscles by Sutherland and Rall, named adenil-cyclase. It is made up of one molecule of adenosine-triphosphate in the presence of Mg ions and the absence of one pyrophosphate molecule. Another enzyme discovered by Butcher and Sutherland hydrolyzes the cyclic AMP into linear AMP. As it hydrolyzes the "diesterea" bond the enzyme has been classified among the "phosphodiesterase".

Control of Cyclic AMP

The level of such a substance noticeably increases in the liver and the muscles when treated with epinephrine, and in vitro with the addition of glucagon (Robison, Butcher, Sutherland). Now we come to the fundamental point which we proposed. The concentration of cyclic AMP usually increases with treatment of methyl-xantine and particularly

with caffeine (Butcher and Sutherland). We have tried to clear up which relations exist between the increased cyclic AMP, the activity of phospherylase, and the effect of caffeine. The problem has been solved since Walsh, Perkins, and Krebs have isolated and purified 200 times another enzyme that provokes the phospherylation of the kinase responsible for the addition of 2 phospherylase b halves (inactive) in the active tetramer. The cyclic AMP is activated allosterically and to distinguish it from the others, the authors have named it cyno-kinase. We can now schematically reconstruct the enzymatic reactions and the effects that modify it, representing it in such a way as to visualize the physiological succession of its concatenated actions, as it is likely to happen in the liver and the muscles.

The scheme is shown in Figure 2, and shows the succession of the reactions which can be compared to a cascade.

We start with the epinephrine (and we also add the glucagon) which activates the adenil-cyclase, which increases the concentration of cyclic AMP. This metabolite seems to be responsible for the development of all successive reactions, It produces allosteric activity of the cyno-kinase or of the enzyme which acts on the kinase responsible for the transformation of phospherylase b into form a, or active. In this sense cyclic AMP is considered to be an allosteric effector from the metabolic point of view, in the control of the complex mechanism of glycogenolysis.

Additive Control of Caffeine

If we now insert in the cascade mechanism the effect brought about by caffeine, we notice that, inhibiting competitively the specific phosphodiesterase that hydrolyses the cyclic AMP, it produces with an inverted mechanism and therefore with a negative control, the same effect produced by epinephrine that acts as a positive control on the adenil-cyclase. This explains the glycogenolytic-synergic effect exerted in vivo by epinephrine and caffeine. and The action of the chemical mediator of information exercised by cyclic AMP seems amplified. It appears that the same cyclic AMP increase is obtained whether by exercising a positive control on the adenil-cyclase through the epinephrine (and glucagon) or a negative control on the phosphodiesterase through caffeine. The proposed model shows that even caffeine can participate in the regulatory mechanism of a fundamental enzyme such

as phospherylase (the Angle-Saxons call them Key enzymes) in what influences the metabolic sequence of primary physiological importance such as glycogenolysis. Therefore, we can conclude that some drugs exert their action by participating in the allosteric variations of key enzymes placed before the fundamental metabolic controls. Generalizing and remembering that even the heart is a muscle, even the positive inotropic effect of caffeine can be attributed to the metabolic control exerted on the phosphodiesterase in that it produces an increase in the concentration of cyclic AMP.

Lipid Metabolisms

It has been known that the triglycerides which make up the fatty tissue eliminate the lypolitic response of various enzymes, such as epinephrine, glucagon, ACTH (adrene-cortical), TSH (thyroid), and others (Butcher). More recent are the observations of Butcher and colleagues that the lypolitic effect of catecholamines is mediated by the increase of cyclic AMP. The presence of an adenil-cyclase sensitive to epinephrine has been demonstrated first in a soluble preparation of fatty tissue. Later it was demonstrated that caffeine acts synergetically with epinephrine as seen in the activity of a lipase extracted from the epididymal fatty deposits of rats, which was stimulated by the addition of cyclic AMP. An example of synergism of epinephrine and caffeine in the increased production of fatty acids (FFA) parallel to the increase of cyclic AMP has been shown in Figure 3, obtained from the data of Butcher and colleagues.

They show very clearly that the phenomenon of lipolysis is correlated to the increase in the level of cyclic AMP. The negative facts obtained adding artificial cyclic AMP to a preparation of non-soluble testicular fatty deposits have been interpreted in the sense that the effector has not been put in contact with the enzymatic system on which it is supposed to act. Adding instead the dibutyryl-cyclic" derivatives of the soluble cyclic AMP on the lipids, the effect is re-established. All these results were recently confirmed determining the speed of glycerol formation as a means of indicating the intensity of the lipolysis.

Terminal Metabolism

There is no direct experience that the addition of cyclic AMP will produce an increase of mitochondrial O_2 even if the adenil cyclase is located in the mitochondrial membrane near all the other enzymes responsible for oxidized phospherylase. Nor is there research proving

the effects of caffeine on such enzymes. The old observation of the stimulus of respiration brought on by caffeine can be explained with an indirect effect due to the accumulation of metabolites coming from the oxidative destruction whether of lactic acid of fatty acid, whose increase we have noted is controlled by the caffeine. The oxidation of either lactic acid or fatty acid produces an increase of acetyl-CoA, which condenses with the oxalacetic acid in the citric cycle, and is completely oxidized by H_2O and CO_2, paving the way for the next intervention of a series of interdependent mitochondrial enzymes and associated with the phospholipids which make up the membranes of the internal matrix of the mitochondria, known as the respiratory chain. This way the O_2 is consumed, the H_2O is produced and the enormous quantity of energy (reduced during the oxidation cycle) is made available in the form of energy associated with the synthesis of AMP. It is clear then that an increase of speed of the aforementioned reaction could explain the calorigenic effect whether of caffeine or the lipolytic hormones.

We are pleased to mention some research which demonstrates how the inhibiters of the oxidative phosphorylation cancel not only the effects of the lipolytic hormones but also of caffeine. Such a result agrees with the need for ATP in the transformation of cyclic AMP to stimulate lipolytic activity.

Concluding Observations

The results regarding the competitive inhibitions exerted by caffeine on the activity of the phosphodiesterase responsible for the hydrolisis of cyclic AMP in the muscles and the liver have been essential in attributing to the drug properties similar to those of several cellular metabolites capable of interacting with the enzymatic proteins, modifying the activity. The inhibition of the phosphodiesterase, which increases the cyclic AMP, starts a series of reactions catalyzed by specific enzymes, among which is one catalyzed by the kinase activated by the cyclic AMP, which has been shown essential in regulating the transformation of inactive phosphorylase b in its form of greatest attraction, into active phosphorylase a. Caffeine, like epinephrine, which acts on other enzymes, has thus shown itself capable of controlling the glycogenolysis and the glucidic metabolism.

Also important are the results of lipolytic effects. The mechanism is similar to the preceding one of caffeine and epniephrine, although here with different mechanisms both produce cyclic AMP increases which activate the lipase responsible for increasing the free fatty acids. This mixture together with the metabolites coming from the glycolysis, converge in one single metabolite, acetyl Co A whose increased production

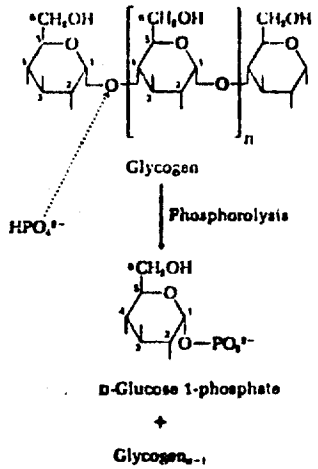
stimulates an increase in the citric cycle and therefore of the oxidative phosphorylation.

The mechanisms illustrated above explain the lipolytic effect, the increase of lactic acid, hyperglycemia, and the consumption of O_2 produced in higher animals following the administration of caffeine. Regarding the pharmacological mechanism in general, the model proposed by us affirms that all drugs capable of provoking a statistically significant variation of the cyclic AMP concentration are also capable of exerting additive metabolic control on that produced by the common cellular metabolites on those enzymes that are structurally adapted to endure the allosteric controls.

BIBLIOGRAFIA

1) Stadtman E. R.: Adv. in Enzymology, 28, 41, 1966.
2) Cori C. F., Cori G. T., Green A. A.: J. Biol. Chem., 151, 39, 1943.
3) Seery V. L., Fischer E. H., Teller D. C.: Biochemistry, 6, 3315, 1967.
4) De Vincenzi D. L., Hedrick J. L.: Biochemistry, 6, 3489, 1967.
5) Fischer E. H., Pocker A., Stein E. C.: Essay in Biochemistry, 6, 23, 1970.
6) Krebs E. G., Fischer E. H.: Biochem. Biophys. Acta, 20, 150, 1956.
7) Baranowski T. et Al.: Biochim. Biophys. Acta, 25, 16, 1957.
Snell E. E.: Vitamin Hormon, 16, 77, 1958.
8) Brunice T. C., Topping R. M.: J. Am. Chem. Soc., 85, 1480, 1963.
9) Keller P. J., Cori G. T.: J. Biol. Chem., 214, 127, 1955.
Merlevede W., Riley G. A.: J. Biol. Chem., 241, 3517, 1966.
Hurd S. S., Ph. D. Thesis: University of Washington, 1967.
10) Hurd S. S., Teller D., Fischer E. H.: Biochem. Biophys. Res. Commun., 24, 79, 1966.
Fischer E. H. et Al.: In Whelan W. J.: «Control of glycogen metabolism», pag. 19. Proc. 4th Meeting of the F.E.B.S., Oslo, 1968. Ed. Academic Press, London.
11) Cori C. F., Cori G. T.: Proc. Soc. Exp. Biol. Med., 34, 702, 1936.
Farnas M. J. K., Mochnacka I.: C. r. Soc. Biol., 123, 1123, 1936.
12) Monod J., Changeux J. P., Jacob F.: J. Mol. Biol., 6, 306, 1963.
13) Monod J., Wyman J., Changeux J. P.: J. Mol. Biol., 12, 88, 1965.
14) Koshland D. E., Nemethy G., Filmer D.: Biochemistry, 5, 365, 1966.
Koshland D. E., Neet K. E.: Ann. Rev. Biochem., 37, 359, 1968.
15) Holzer H.: Adv. in Enzymology, 32, 297, 1969.
16) Sutherland E. W., Rall T. W.: Pharmacol. Rev., 12, 265, 1960.
17) Rall R. W., Sutherland E. W.: J. Biol. Chem., 237, 1228, 1962.
18) Butcher R. W., Sutherland E. W.: J. Biol. Chem., 237, 1244, 1962.
19) Robison G. A., Butcher R. W., Sutherland E. W.: Ann. Rev. Bioch., 37, 149, 1968.
20) Walsh D. A., Perkins J. P., Krebs E. G.: Fed. Proc., 27, 339, 1968.
21) Butcher R. W.: Pharmacol. Rev., 18, 237, 1966.
22) Butcher R. W., Ho R. J., Meng H. C., Sutherland E. W.: J. Biol. Chem., 240, 4515, 1965.
23) Vaughan M., Steinberg D. J.: Lipid Res., 4, 193, 1963.
24) Rizack M. A.: J. Biol. Chem., 239, 392, 1964.
25) Butcher R. W., Robison G. A., Hardman J. G., Sutherland E. W.: Adv. Enz. Regul., 6, 357, 1968.
26) Heim T., Hull D.: J. Physiol., 187, 271, 1966.
27) Fassina G.: Life Sci., 6, 825, 1967.

Phosphorolytic removal of a glucose residue from the nonreducing end of a glycogen chain by phosphorylase.



Conversion of phosphorylase a to phosphorylase b by phosphorylase phosphatase and reactivation of phosphorylase b by phosphorylase kinase

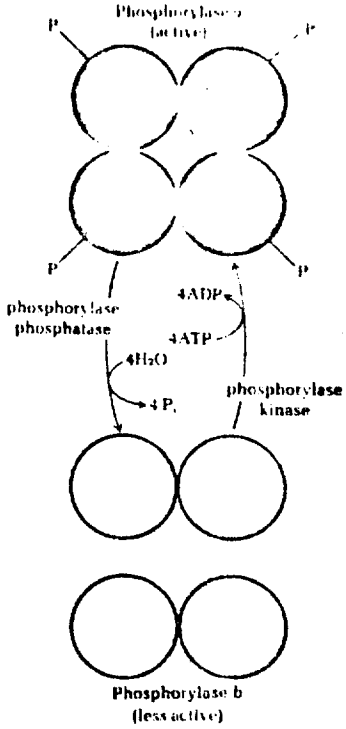


Figure 1.

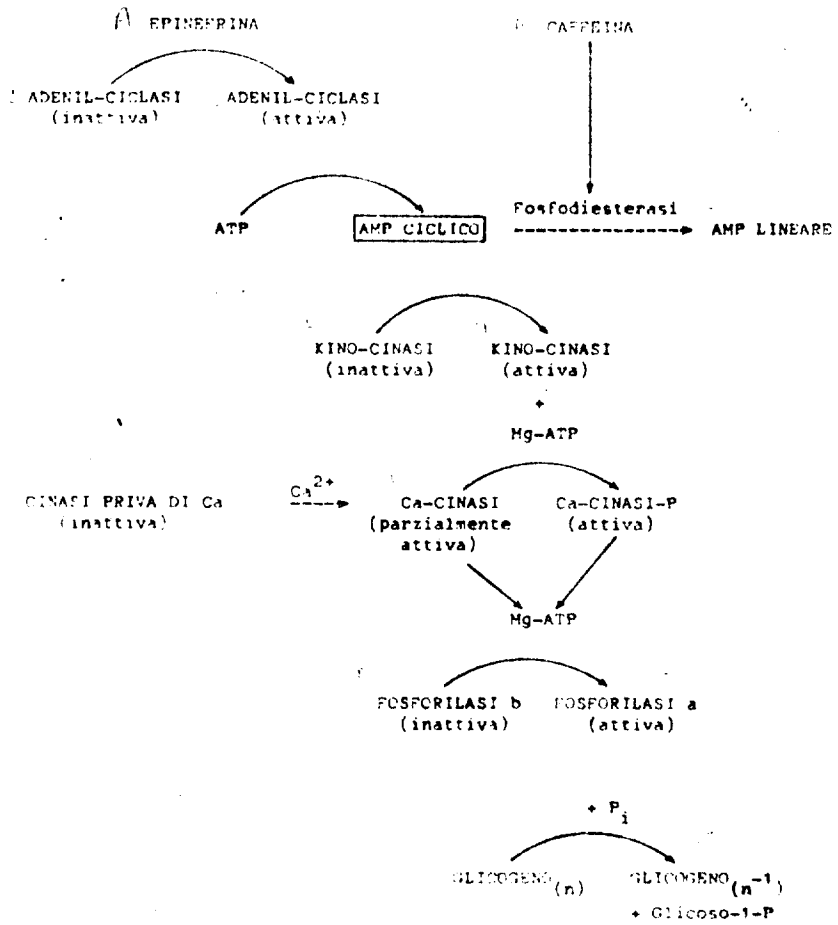


Figure 2. Control of cyclic AMP in the cascade reaction which activates the phosphorylase. Key: A-epinephrine, B-caffeine, C-adenil-cyclase (inactive), D-adenil-cyclase (active), E-cyclic AMP, F-Phosphodiesterase, G-linear AMP, H-cyno-kinase inactive, I-cyno-kinase active, J-kinase without Ca (inactive), K-Ca Kinase partially active, L-Ca Kinase-P active, M-Phosphorylase b inactive, N-Phosphorylase a active, O-glycogen, and P-glycogen.

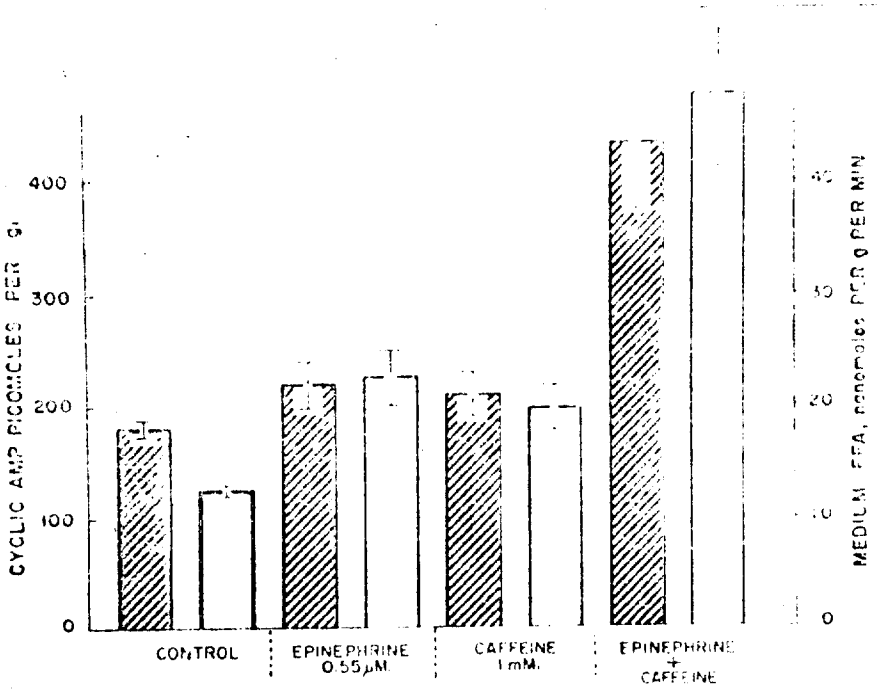


Figure 3. Synergism between epinephrine and caffeine on the cyclic AMP level and the freeing of fatty acids in the epididymal adipose tissue of rats.

Questions to Professor Ruffo

Prof. Fidanza

Professor Ruffo, what would be the participation of caffeine, or better yet, of coffee, on the energy wear of an individual? Have you any data? It participates at a metabolic level, as we have seen, in determined conditions, but does it participate also in the energetic metabolism of the individual?

Professor Ruffo

Prof. Fidanza has asked the question in very restricted terms: it requires quantitative facts. It is clear that I have spoken of an effect at a molecular level, and it is also clear that we must expect that a certain quantity of glucose is going to be produced. But let us understand - it is not a qunatity which would produce an appreciable hyperglycemia, such as a diabetic one, it only makes the glucose appear more noticeable, as seen in animals, and which also might be found in men. As for the eventual participation of caffeine, on the basal metabolism, that is, will it produce an increase in the basal metabolism of man I would not want to say at this point. I think not, but I am not really sure.

Prof. Zambetti

My question deals with the inhibiting action of caffeine on the phosphodiesterase. I would like to have, if possible, any quantitative facts on this interesting inhibiting action. I would also like to know if this same action occurs with methyl xantine, theophylline, and theobromine, and other pure derivatives.

Various experiments are being done to introduce cyclic adenil acid in therapy. One of the obstacles to overcome is the speed with which the substance is dissolved in the phosphodiesterase. I would like to know Prof. Ruffo's opinion on the very real possibility of increasing the stability of the adenil acid in vivo through the use of caffeine, a similar substance, or other substances, in order to allow it to perform an effective therapeutic action.

Prof. Ruffo

Yes, as a hypothesis it is acceptable, as well as brilliant, and we should recommend it to the pharmaceutical chemists who would work in this area. That cyclic AMP has been introduced in therapy is a fact that I think is now discounted in the literature. There have been

many experiments on the butyrilic derivatives. The cyclic AMP molecule tends to stabilize when by introducing into the amine group the radicals of fatty acids in a short chain, which give (we do not know for what stearic reason) the mixture a better stability.

From the general point of view, more than just a drug, cyclic AMP has become a precious substance for transmitting information within the cell.

It is obvious that the problem, suggested by Prof. Zambetti, of administering caffeine and AMP together represents a fact which should be confirmed by experiments. I do not think it would be considered lost time to perform such experiments.

Here we agree with Prof. Zambetti: this is obviously an open field, one of great interest, and we hope that experimental data will be found which will clarify the biological workings of such drugs.

Prof. Paoletti

Certainly the inhibitng action of caffeine on the phosphodiesterase is one of the most important mechanisms because of its pharmacological action. I would still like to clarify that the phosphodiesterase are different from tissue to tissue, and that caffeine is among the physiological (or better, natural) inhibiters that best inhibits the cerebral phosphodiesterase. The theophylline is not capable of inhibiting the phosphodiesterase of the brain, and this might greatly explain the difference between coffee and tea at a central level. The theophylline blocks more actively than the caffeine, at the renal level, and is also relatively active at the myocardial level, although at that level the caffeine is more active.

The theobromine is moderately active in the nervous system, not too active at the renal level, and very strong at the myocardial level. This difference is due to differences already noted, of physical chemistry properties of these enzymes, which are not similar but vary from organ to organ. In the nervous system, two or three different types of phosphodiesterase are added, of which only one is blocked by the caffeine and the others are not.

It is clear therefore that caffeine can be very active in altering and modifying the action of cyclic AMP in the tissue, in making the action of cyclic AMP more specific, because it prolongs life in some tissues and not in others. If this is a positive or negative fact depends on the circumstances.

I think it is a very active drug, which must be studied accurately in the

newborn, and in the fetal tissue where evidently we would not want the effects of cyclic AMP strengthened. We must remember that the adenil cyclase enzyme, which modulates the synthesis of cyclic AMP is present in the fetal tissue before the sympathetic or para-sympathetic innervation. It is an enzyme that pre-exists during entogenesis as a control of the sympathetic system as an activator, or of the para-sympathetic as an inhibiter.

When this enzyme is found present, activated by a variety of hormones but not exactly controlled, it is not useful in prolonging the average survival of cyclic AMP. In some cases the effect can be favorable when for example we are in the presence of stress, relatively brief in time, which strongly engages the energy reserves, and this of course we all knew through the use of caffeine in sports.

I also believe that caffeine should be considered not only a very active drug, but also a selective one, a drug which must be better known, especially its dosage. I do not find it acceptable what has occured today, that is, having a mixture of various types of coffee or various forms of coffee, in which the quantity of caffeine is unknown to those who have had some. If we are leaning towards a greater control of food, from the pharmacological point of view, we have the duty and the right to know the doses of these potent drugs which we ingest, for our own good, and to avoid illness, which could always happen.

I would like to recall that the effect of caffeine on the nervous system was first explored by Moresini, who was the delegate of the Venetian Republic to Cyprus. In his letter of 1570 he suggested to the Venetians the use of cave, the Turkish name used in Hungary and other countries for coffee, because says he, "it has men aroused", and he would give it to his co-workers. Evidently there was already the feeling of artificial stimulus in his co-workers.

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MINERVA MEDICA

RELAZIONI

Relazioni tra caffeina e metabolismo intermedio

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La composizione chimica delle cellule viventi, malgrado il continuo modificarsi provocato dalle varie reazioni metaboliche, è mantenuta costante da delicati meccanismi di controllo dell'attività enzimatica capaci di produrre modificazioni di intere sequenze metaboliche. Lo studio di tale argomento si è arricchito in questi ultimi anni di una serie di dati sperimentali che hanno permesso di considerare un nuovo aspetto della fisiologia cellulare, quello della *regolazione metabolica*.

Il termine fu coniato dapprima per spiegare il caratteristico aumento della respirazione mitocondriale prodotto dall'ADP (adenosin-difosfato) che determina un aumento del consumo di O_2 e dei substrati ossidabili, tale da spostare la respirazione mitocondriale dal suo stato di riposo, contraddistinto dalla sigla *stato IV*, ad una condizione circa 10 volte più attiva, corrispondente al cosiddetto *stato III*. La regolazione è specifica e scompare quando tutto l'ADP si è trasformato, tanto che il fenomeno è ormai noto in biochimica con il nome di *controllo respiratorio*, e contribuisce a spiegare anche altri fondamentali correlazioni metaboliche, quale l'inibizione della glicolisi prodotta dall'O_2, scoperta da Pasteur. Più recentemente sono stati messi in evidenza altri tipi di regolazione, fra cui quella detta *allosterica* perchè dipendente dall'effetto di particolari metaboliti che agiscono su siti non-catalitici degli enzimi, detti appunto allosterici, e che in genere si accompagnano a modificazioni strutturali delle molecole enzimatiche [1].

Scopo del presente lavoro è quello di tentare di spiegare il meccanismo d'azione di un farmaco, quale la caffeina, sulla base di una sua possibile interazione con enzimi connessi con la regolazione allosterica.

Metabolismo glucidico

Sono ormai di acquisizione classica i vecchi dati secondo cui l'iniezione di caffeina in animali superiori produce iperglicemia, associata ad aumento della glicolisi e del consumo di O_2. Nel muscolo isolato la produzione di acido lattico è tanto elevata da portare alla contrattura. Tutti questi risultati avevano già fatto pensare che il farmaco dovesse agire a livello dei sistemi enzimatici responsabili dell'aumento sia del glucosio sia dell'acido lattico e cioè a livello della fosforilasi. Però si sapeva troppo poco di quest'enzima assai complesso per impostare la questione nei suoi esatti termini, salvo che una maggiore attività della fosforilasi nel fegato, a causa della presenza dell'esoso-fosfatasi, provocava aumento della produzione del glucosio, che si versava nel sangue, mentre nel muscolo, dove non è contenuta la fosfatasi, permetteva una maggiore produzione dell'acido lattico, in quanto veniva aumentata la demolizione anaerobica del glucoso-1-fosfato.

Natura della fosforilasi

Oggi è ben noto che la fosforilasi è uno di quegli enzimi che oltre ai siti catalitici presenta siti detti *regolatori* perchè hanno importanza per interagire con composti semplici, in genere metaboliti, capaci di modificare la velocità enzimatica in modo tale da far variare l'andamento di un'intera sequenza metabolica. L'enzima estratto dai muscoli scheletrici da Cori, Cori e Green nel 1943 [2] è una proteina cristallina [3] dal peso molecolare di 380.000 [4], corrispondente ad un tetramero costituito da 4 monomeri pressochè uguali fra di loro [5].

Ciascun monomero presenta un residuo di fosfoserina essenziale per l'attività catali-

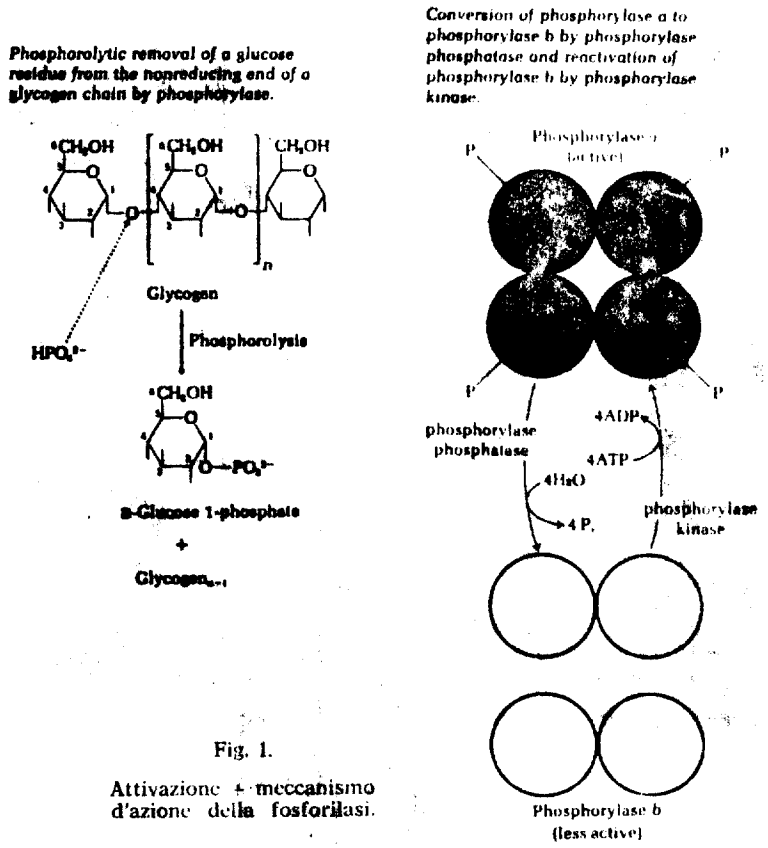

Fig. 1.

Attivazione e meccanismo d'azione della fosforilasi.

tica [6], ed una molecola di piridossalfosfato [7] legato covalentemente ad un residuo di lisina [8]. Solo quando l'enzima si trova in tale conformazione strutturale prende il nome di fosforilasi *a* ad indicare che è capace di trasformare l'estremità terminale di una molecola di glicogeno (con un meccanismo trasferente su di un radicale di fosfato inorganico) in una molecola di glucoso-1-fosfato ed in un residuo di glicogeno inferiore di una unità.

La fig. 1 mostra che quando la forma attiva viene attaccata da un enzima idrolitico anch'esso presente nel muscolo, una fosfatasi specifica [9], che idrolizza i 4 residui fosforici legati ai 4 residui di serina, l'enzima si inattiva. L'inattivazione corrisponde alla rottura dell'unità tetramera in due dimeri di peso molecolare metà dei precedenti, incapaci di attaccare il glicogeno e che per distinguerli dal precedente, prendono il nome di fosforilasi *b* (inattiva). La reazione è reversibile, ma catalizzata da un altro enzima, ugualmente presente nel muscolo, la fosforilasi *b* cinasi [10] che ha bisogno di Ca, Mg ed ATP. E' chiaro che in tal modo la concentrazione di fosforilasi attiva nelle cellule può variare ed in conseguenza l'entità della glicogenolisi. Anche nel fegato sono state trovate le due forme *a* e *b* della fosforilasi, ma la loro struttura è parzialmente differente, così come il meccanismo che ne determina l'attività.

Attivazione della fosforilasi

Le prime osservazioni riguardano l'effetto esercitato dall'AMP sulla fosforilasi *b* che in presenza di tale nucleotide diventa attiva [11]. Da allora ad oggi una notevole quantità di dati ha permesso a Monod e Coll. prima di prevedere [12] e poi di dimostrare [13] che la fosforilasi subisce un'attivazione allosterica con conseguente cambiamento conformazionale. Tuttavia non è possibile per ora prospettare un modello più preciso di quello semplificato presente in fig. 1, perchè sul processo agiscono diversi fattori che complicano il meccanismo dell'aggregazione [14]. Soprattuto modificano le curve carat-

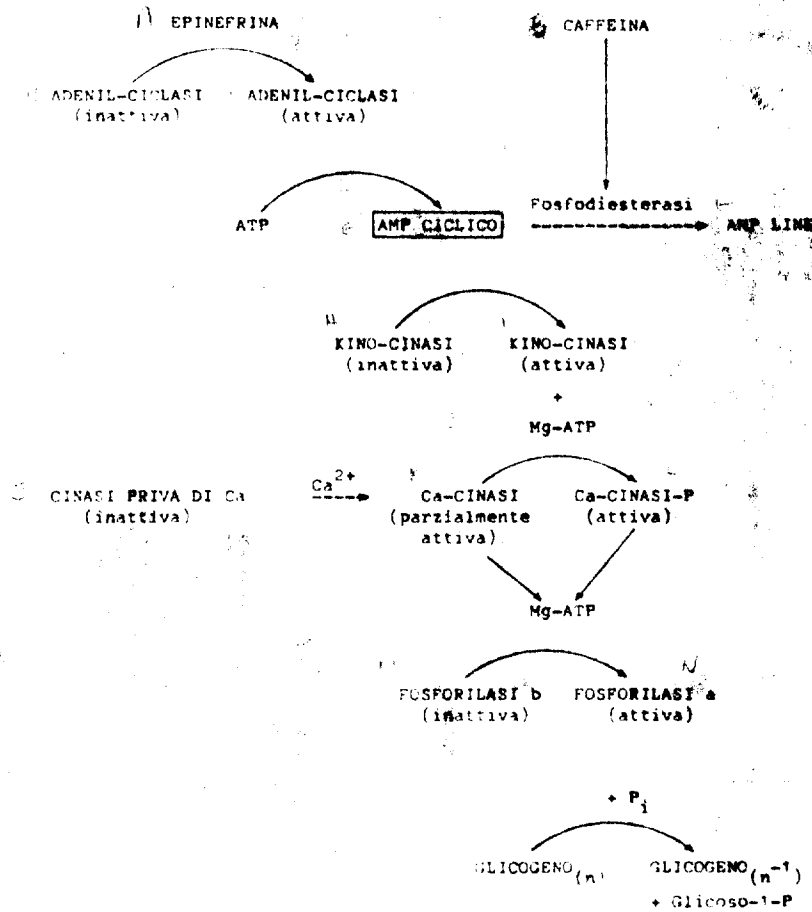

Fig. 2. — Regolazione da AMP-ciclico delle reazioni a *cascata* che *attivano* la fosforilasi.

teristiche della cinetica che esprime il processo di attivazione allosterica, l'intervento di macromolecole enzimatiche capaci a loro volta di produrre modificazioni strutturali su altri enzimi. Una condizione chimico-fisica assolutamente nuova alla quale sono paragonabili solo altri rari esempi di interazioni fra macromolecole, quali l'attivazione della glutamina sintetasi ed, in certo senso, l'attivazione degli enzimi proteolitici [15] connessi con la coagulazione del sangue.

Confrontando i numerosi risultati ottenuti in questo settore con altri ugualmente fondamentali ottenuti in campo fisiologico, quali quelli relativi all'effetto dell'epinefrina (adrenalina) e del glucagone nel produrre iperglicemia, un nuovo campo d'indagine si è aperto allo scopo di correlare i rapporti fra ormoni ed iperglicemia col meccanismo d'azione dei nuovi enzimi preposti alla attivazione della fosforilasi epatica. Tali studi hanno portato alla identificazione di un nuovo metabolita, il cosiddetto AMP-ciclico con un mediatore chimico dell'effetto ormonale ed, in qualche caso, anche dell'impulso nervoso [16].

L'AMP-ciclico è una particolare forma diesterea (precisamente 3'-5'-fosfato) di un nucleotide ben noto ed assai diffuso in tutte le cellule l'adenosin-5'-fosfato, il quale ad opera di un enzima scoperto da Sutherland e Rall nei muscoli [17] detto adenil-ciclasi, si forma da una molecola di adenosin-trifosfato in presenza di ioni Mg e con allontanamento di una molecola di pirofosfato. Un altro enzima scoperto anch'esso da Butcher e Sutherland [18] idrolizza l'AMP-ciclico in AMP-lineare: siccome idrolizza il legame diestereo l'enzima è stato classificato fra le fosfodiesterasi.

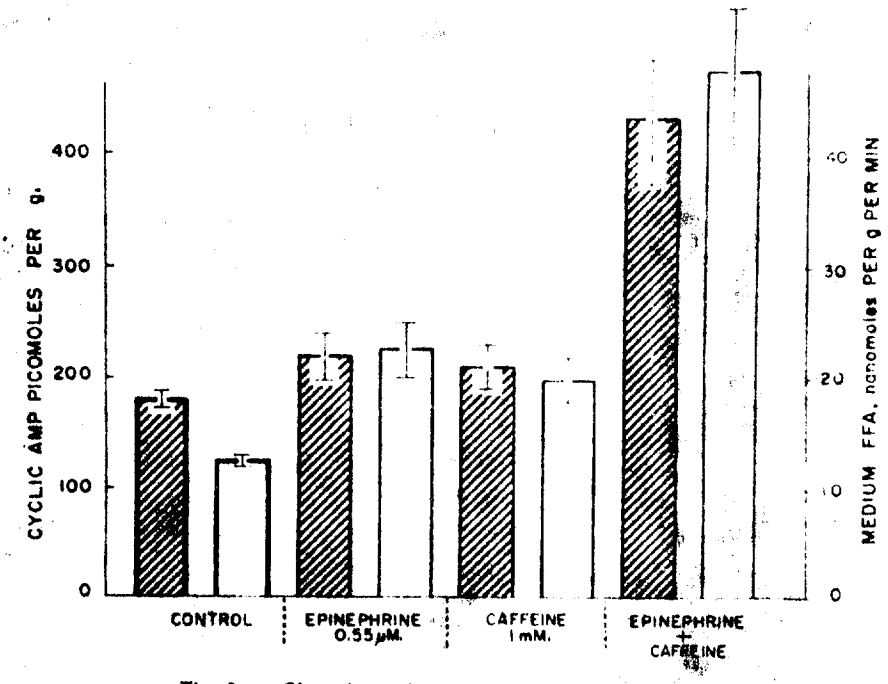

Fig. 3. — Sinergismo fra epinefrina e caffeina sui livelli di AMP-ciclico e la liberazione di acidi grassi liberi nel tessuto adiposo epididimale del ratto.

Controllo da AMP-ciclico

Il livello di tale sostanza aumenta notevolmente nel fegato e nel muscolo per trattamento con epinefrina ed *in vitro* per aggiunta anche di glucagone (Robison, Butcher e Sutherland[19]). Ed ora veniamo al punto fondamentale che ci eravamo proposti: la concentrazione di AMP-ciclico aumenta ugualmente per trattamento con metil-xantine ed in particolare di caffeina (Butcher e Sutherland, l.c.[18]). Cerchiamo dunque di chiarire quali relazioni esistono fra l'aumento di AMP-ciclico, l'attivazione della fosforilasi ed effetto da caffeina. Il problema è stato risolto in questi ultimi tempi dopo che Walsh, Perkins e Krebs[20] hanno isolato e purificato 200 volte un altro enzima che provoca la fosforilazione della cinasi responsabile dell'aggregazione dei 2 dimeri di fosforilasi *b* inattivi nel tetramero attivo. Su di esso agisce l'AMP-ciclico attivandolo allostericamente e, per distinguerlo dai rimanenti, gli Autori lo hanno chiamato *cino-cinasi*. Siamo in condizione ora di ricostruire schematicamente le reazioni enzimatiche e gli effettori che le modificano, rappresentandoli in modo tale da visualizzare la successione fisiologica delle loro azioni concatenate, come è verosimile che avvenga nel fegato e nel muscolo, quando si verifica glicogenolisi.

Lo schema è riportato in fig. 2 e mostra la successione delle reazioni che si possono paragonare alla caduta di una « cascata ».

L'avvio è dato dalla epinefrina (ed aggiungiamo anche dal glucagone) che attiva l'adenil-ciclasi, che fa aumentare la concentrazione di AMP-ciclico. Questo metabolita sembra essere il responsabile del decorso di tutte le reazioni successive, producendo attivazione allosterica della cino-cinasi, ovvero di quell'enzima risultato specifico nell'agire sulla cinasi responsabile della trasformazione della fosforilasi *b* nella forma *a*, attiva. In questo senso l'AMP-ciclico viene considerato un *effettore allosterico* nel regolare, dal punto di vista metabolico il complesso meccanismo della glicogenolisi.

Controllo additivo della caffeina

Se inseriamo adesso nel meccanismo a « cascata » l'effetto esercitato dalla caffeina, ci accorgiamo come essa, inibendo competitivamente la fosfodiesterasi specifica che

idrolizza l'AMP-ciclico, produce, con un meccanismo inverso, e cioè con un controllo *negativo*, lo stesso effetto prodotto dall'epinefrina che agisce con un controllo *positivo* sull'adenil-ciclasi. In tal modo si spiega l'effetto sinergico glicogenolitico esercitato *in vivo* da epinefrina e caffeina, ed appare ampliata in maniera assai concreta l'azione di mediatore chimico d'informazione esercitato dall'AMP-ciclico. Appare infatti che lo stesso aumento di AMP-ciclico si ottiene sia esercitando un controllo positivo sull'adenil-ciclasi, tramite l'epinefrina (ed il glucagone) sia un controllo negativo sulla fosfodiesterasi, tramite la caffeina. Il modello prospettato mette in evidenza come anche la caffeina possa partecipare al meccanismo di regolazione di un enzima fondamentale come la fosforilasi (gli anglosassoni li chiamano enzimi «chiave») in quanto presiede ad una sequenza metabolica di primaria importanza fisiologica quale la glicogenolisi. Pertanto sembra di poter concludere che alcuni farmaci esercitano la loro azione partecipando, insieme ai metaboliti cellulari, alla modulazione allosterica di enzimi «chiave» preposti a fondamentali regolazioni metaboliche. Generalizzando e ricordando che anche il cuore è un muscolo, anche l'effetto inotropo positivo della caffeina si può attribuire alla regolazione metabolica esercitato sulla fosfodiesterasi in quanto produce aumento della concentrazione di AMP-ciclico.

Metabolismo lipidico

Che i trigliceridi costituenti il tessuto adiposo subissero una risposta lipolitica all'effetto di vari ormoni, quali l'epinefrina, il glucagone, l'ACTH (adreno-corticale), TSH (tiroideo), ed altri, era noto da tempo (Butcher [illegible]). Più recente invece l'osservazione di Butcher e Coll. [illegible] che l'effetto lipolitico delle catecolamine, è mediato dall'aumento dell'AMP-ciclico. La presenza di una adenil-ciclasi sensibile all'epinefrina venne dimostrata dapprima in preparazioni solubilizzate di tessuto adiposo (l.c. [illegible]) e poi riuscì a dimostrare che la caffeina agiva sinergicamente con l'epinefrina [illegible] in quanto l'attività di una lipasi estratta dai depositi di grasso condizionati di ratto veniva stimolata dall'aggiunta di AMP-ciclico [illegible]. Un esempio del sinergismo dell'epinefrina e della caffeina nel produrre aumento della produzione di acidi grassi liberi (FFA) parallelo all'aumento di AMP-ciclico, è riportato nella fig. 3, ricavata dai dati di Butcher e Coll. (l.c. [22]).

Essi mostrano molto chiaramente come il fenomeno della lipolisi sia correlato all'aumento del livello di AMP-ciclico. Quei dati negativi ottenuti aggiungendo artificialmente AMP-ciclico a preparazioni di depositi grassi testicolari non solubilizzate, vanno interpretati nel senso che l'effettore non è riuscito a giungere in contatto con i sistemi enzimatici su cui doveva agire. Infatti aggiungendo invece derivati dibutirrilici dell'AMP-ciclico solubili nei lipidi, l'effetto si ripristina. Tutti questi risultati sono stati recentemente confermati [25] determinando la velocità di formazione del glicerolo come mezzo indicante l'intensità della lipolisi.

Metabolismo terminale

Non si ha finora esperienza diretta che l'aggiunta di AMP-ciclico produca incremento del consumo di O_2 mitocondriale, anche se l'adenil-ciclasi è stata trovata localizzata nelle membrane mitocondriali insieme a tutti gli altri enzimi responsabili delle fosforilazioni ossidative; nè si ha conoscenza di ricerche comprovanti, l'effetto della caffeina su tali enzimi. Tuttavia la vecchia osservazione dello stimolo della respirazione provocata dalla caffeina si può spiegare bene con un effetto indiretto dovuto all'accumulo di metaboliti provenienti dalla demolizione ossidativa sia dell'acido lattico sia degli acidi grassi, il cui aumento, come abbiamo visto, è sotto il controllo della caffeina. Com'è ben noto l'ossidazione sia dell'acido lattico sia degli acidi grassi produce aumento di acetil-CoA che, entrando in seguito a condensazione con l'acido ossalacetico nel ciclo citrico, viene completamente ossidato ad H_2O e CO_2, tramite il successivo intervento di una serie di enzimi mitocondriali interdipendenti ed associati ai fosfolipidi costituenti le membrane della matrice interna dei mitocondri noti col nome di *catena respiratoria*. In tal modo si consuma O_2, si produce H_2O e si rende utilizzabile la grande quantità di energia degradata nel corso dell'ossidazione degli intermediari del ciclo, sotto forma di energia associata alla sintesi di AMP. E' chiaro dunque come un aumento della velocità delle suddette reazioni possa spiegare l'effetto calorigeno sia della caffeina, sia degli ormoni lipolitici [26].

A tale proposito ci piace segnalare una ricerca [27] che mostra come gli inibitori della

fosforilazione ossidativa annullano non solo l'effetto degli ormoni lipolitici ma anche quello della caffeina. Tale risultato è in accordo con il bisogno di ATP necessario a trasformarsi in AMP-ciclico per stimolare l'attività lipolitica.

Osservazioni conclusive

Il risultato relativo all'inibizione competitiva esercitata dalla caffeina sull'attività della fosfodiesterasi responsabile dell'idrolisi dell'AMP-ciclico nei muscoli e nel fegato è stato fondamentale per attribuire al farmaco proprietà simili a quelle di vari metaboliti cellulari capaci di interagire con le proteine enzimatiche modificandone l'attività. L'inibizione della fosfodiesterasi, provocando aumento di AMP-ciclico, mette in moto una serie di reazioni catalizzate da enzimi specifici, fra cui una catalizzata dalla cinasi attivata allostericamente dall'AMP-ciclico, che si è dimostrata fondamentale nel regolare la trasformazione della fosforilasi *b* inattiva nella sua forma di maggiore aggregazione, la fosforilasi *a* attiva. In tal modo la caffeina si è mostrata capace, così come l'epinefrina (che però agisce su altri enzimi) di regolare la glicogenolisi e quindi il metabolismo glucidico.

Altrettanto importanti i risultati relativi all'effetto lipolitico. Il meccanismo è paragonabile al precedente in quanto caffeina ed epinefrina, anche qui con meccanismi differenti, entrambi producono aumento di AMP-ciclico che attiva le lipasi responsabili dell'aumento degli acidi grassi liberi. Tali composti, insieme ai metaboliti provenienti dalla glicolisi, convergono in un unico metabolita, l'acetil-CoA, la cui aumentata produzione provoca incremento del ciclo citrico e con esso della fosforilazione ossidativa.

I meccanismi sopra illustrati spiegano sia l'iperglicemia, sia l'effetto lipolitico, sia l'aumento dell'acido lattico e del consumo di O_2 prodotti negli animali superiori in seguito a somministrazione di caffeina. Per quanto poi riguarda il meccanismo farmacologico in generale, il modello da noi prospettato permette di affermare che tutti quei farmaci, che sono capaci di provocare una variazione della concentrazione di AMP-ciclico statisticamente significativa, sono anche capaci di esercitare un controllo metabolico « additivo » a quello prodotto dai comuni metaboliti cellulari su quegli enzimi che sono strutturalmente adatti a subire la regolazione allosterica.

Bibliografia

1) Stadtman E. R.: Adv. in Enzymology, 28, 41, 1966.
2) Cori C. F., Cori G. T., Green A. A.: J. Biol. Chem., 151, 39, 1943.
3) Seery V. L., Fischer E. H., Teller D. C.: Biochemistry, 6, 3315, 1967.
4) De Vincenzi D. L., Hedrick J. L.: Biochemistry, 6, 3489, 1967.
5) Fischer E. H., Pocker A., Saari J. C.: Essay in Biochemistry, 6, 23, 1970.
6) Krebs E. G., Fischer E. H.: Biochem. Biophys. Acta, 20, 150, 1956.
7) Baranowski T. et Al.: Biochim. Biophys. Acta, 25, 16, 1957.
Snell E. E.: Vitamin Hormon, 16, 77, 1958.
8) Bruice T. C., Topping R. M.: J. Am. Chem. Soc., 85, 1480, 1963.
9) Keller P. J., Cori G. T.: J. Biol. Chem., 214, 127, 1955.
Merlevede W., Riley G. A.: J. Biol. Chem., 241, 3517, 1966.
Hurd S. S., Ph. D. Thesis: University of Washington, 1967.
10) Hurd S. S., Teller D., Fischer E. H.: Biochem. Biophys. Res. Comm., 24, 79, 1966.
Fischer E. H. et Al.: In Whelan W. J.: « Control of glycogen metabolism », pag. 19. Proc. 4th Meeting of the F.E.B.S., Oslo, 1968. Ed. Academic Press, London.
11) Cori C. F., Cori G. T.: Proc. Soc. Exp. Biol. Med., 34, 702, 1936.
Parnas M. J. K., Mochnacka I.: C. r. Soc. Biol., 123, 1173, 1936.
12) Monod J., Changeux J. P., Jacob F.: J. Mol. Biol., 6, 306, 1963.
13) Monod J., Wyman J., Changeux J. P.: J. Mol. Biol., 12, 88, 1965.
14) Koshland D. E., Nemethy G., Filmer D.: Biochemistry, 5, 365, 1966.
Koshland D. E., Neet K. E.: Ann. Rev. Biochem., 37, 359, 1968.
15) Holzer H.: Adv. in Enzymology, 32, 297, 1969.
16) Sutherland E. W., Rall T. W.: Pharmacol. Rev., 12, 265, 1960.
17) Rall R. W., Sutherland E. W.: J. Biol. Chem., 237, 1228, 1962.
18) Butcher R. W., Sutherland E. W.: J. Biol. Chem., 237, 1244, 1962.
19) Robison G. A., Butcher R. W., Sutherland E. W.: Ann. Rev. Bioch., 37, 149, 1968.
20) Walsh D. A., Perkins J. P., Krebs E. G.: Fed. Proc., 27, 339, 1968.
21) Butcher R. W.: Pharmacol. Rev., 18, 237, 1966.
22) Butcher R. W., Ho R. J., Meng H. C., Sutherland E. W.: J. Biol. Chem., 240, 4515, 1965.
23) Vaughan M., Steinberg D. J.: Lipid Res., 4, 193, 1963.
24) Rizack M. A.: J. Biol. Chem., 239, 392, 1964.
25) Butcher R. W., Robison G. A., Hardman J. G., Sutherland E. W.: Adv. Enz. Regul., 6, 357, 1968.
26) Heim T., Hull D.: J. Physiol., 187, 271, 1966.
27) Fassina G.: Life Sci., 6, 825, 1967.

INTERVENTI SULLA RELAZIONE DEL PROF. A. RUFFO

Prof. FIDANZA

Prof. Ruffo, quale può essere l'intervento della caffeina, o meglio del caffè, sul dispendio energetico dell'individuo? Avete dei dati? Interviene a livello metabolico, l'abbiamo visto, è vero, in quelle determinate condizioni, ma interviene anche nel metabolismo energetico dell'individuo?

Prof. RUFFO

Il Prof. Fidanza ha messo in termini molto ristretti la questione, vuole un dato quantitativo. È chiaro che io ho parlato di un effetto a un livello molecolare; ed è altrettanto chiaro che ci si attende che una certa quantità di glucosio in più effettivamente si abbia a produrre. Ma intendiamoci, non è una quantità che produca iperglicemia apprezzabile come quella diabetica, non porta alcun scompenso, soltanto fa salire il glucosio verso valori alti, come è stato riscontrato negli animali, ed io credo lo si possa anche riscontrare nell'uomo. Per quanto riguarda l'eventuale intervento della caffeina sul metabolismo basale, se cioè, nell'uomo riesca a provocare un aumento del metabolismo basale non mi vorrei pronunciare su questo punto di pertinenza del fisiologo, penso più no che sì, ma non lo so veramente bene.

Prof. ZAMBOTTI

Il mio breve intervento riguarda l'azione inibente della caffeina sulla fosfodiesterasi. Desidererei avere, se possibile, qualche dato quantitativo su questa interessante azione inibente. Mi piacerebbe inoltre sapere se la stessa azione è [illegible] da altre metaxantine, quali la teoria [illegible] teobromina e da altri derivati purinici.

Si sono [illegible] tentativi per introdurre l'acido adenilico [illegible] in terapia. Uno [illegible] rappresentato dalla [illegible] sostanza è degradata [illegible] fosfodiesterasi. Desidererei [illegible] l'opinione del Prof. Ruffo [illegible] di aumentare la stabilità dell'acido adenilico in vivo mediante la caffeina, sostanze simili o altri composti, in modo da consentire allo stesso di svolgere una effettiva azione terapeutica.

Prof. RUFFO

Sì, in linea di massima l'ipotesi è accettabile e anche brillante, e la potremo consigliare ai chimici farmaceutici, che si occupano di questo argomento. Che l'AMP ciclico sia stato introdotto in terapia credo sia un dato oramai scontato in letteratura. Ci sono stati vari tentativi di certi derivati butirrilici, soprattutto appunto per dare al composto una maggiore stabilità. Inoltre si tende a stabilizzare la molecola di AMP-ciclico introducendo nei gruppi aminici oppure negli ossidrili del riboso questi radicali di acidi grassi a breve catena, che danno — non saprei neanche per quale ragione sterica — una maggiore stabilità al composto.

Tuttavia, credo che dal punto di vista generale, più che un farmaco, l'AMP-ciclico, è risultato essere una sostanza preziosa dal punto di vista della trasmissione dell'informazione, nell'interno delle cellule.

Tuttavia è ovvio che il problema, suggerito da Zambotti, della somministrazione contemporanea di caffeina e AMP rappresenta un dato che dovrebbe essere convalidato dall'esperimento. Io non credo sia tempo perduto quello di fare esperimenti di questo genere.

Quindi sono d'accordo con il Prof. Zambotti: è chiaramente questo un campo di indagine aperto e di estremo interesse e ci auguriamo che forniscano i dati sperimentali che ci permettano di chiarire il meccanismo di questi farmaci ad azione biologica di cui è così difficile mettere in evidenza le proprietà farmacologiche, con i comuni mezzi d'indagine.

Prof. PAOLETTI

Per quanto riguarda l'intervento del Prof. Ruffo, certamente l'azione inibitrice della caffeina sulla fosfodiesterasi è uno dei meccanismi più importanti per la sua azione farmacologica. Vorrei tuttavia sottolineare che le fosfodieste-

rasi sono diverse da tessuto a tessuto e che la caffeina è tra gli inibitori fisiologici o meglio naturali quella che riesce a inibire meglio la fosfodiesterasi cerebrale. La teofillina non è in grado di inibire la fosfodiesterasi del cervello, e questo può spiegare in gran parte la differenza di attività tra caffè e te a livello centrale. Invece la teofillina è un bloccante molto più attivo della caffeina, a livello renale, e anche relativamente attiva a livello del miocardio, dove però la caffeina la supera in attività.

La teobromina ha un'attività modesta sul sistema nervoso, non alta a livello renale e molto forte a livello del miocardio. Questa differenza è dovuta a differenze ben note, di proprietà fisico-chimiche di questi enzimi, che non sono enzimi identici, ma variano da organo ad organo. Nel sistema nervoso vi sono addirittura due o più, forse tre, tipi diversi di fosfodiesterasi, di cui uno solo è bloccato poi dalla caffeina, e gli altri non lo sono.

E' chiaro perciò che la caffeina può essere assai attiva nell'alterare o modificare l'azione dell'AMP-ciclico in qualche tessuto, cioè nel rendere più specifica l'azione dell'AMP-ciclico, perchè ne prolunga la vita in qualche tessuto e non in altri. Ora, se questo sia un fatto positivo o negativo dipende dalle circostanze.

Io penso che sia un farmaco assai attivo, e che debba per esempio essere studiato con grande accuratezza nel neonato, o addirittura nei tessuti fetali, dove evidentemente noi possiamo non desiderare un potenziamento degli effetti dell'AMP-ciclico. Dovremmo ricordarci che l'enzima adenilciclasico, che modula la sintesi dell'AMP-ciclico, è presente nei tessuti fetali prima ancora della innervazione simpatica o parasimpatica; è un enzima che preesiste, durante l'ontogenesi, al controllo del simpatico, come attivatore o del parasimpatico come inibitore.

Quando questo enzima si trova già presente, già attivabile da una varietà di ormoni, ma non esattamente controllato, può essere non utile di prolungarne la sopravvivenza media del prodotto, l'AMP-ciclico. In altri casi, invece, l'effetto può essere senz'altro favorevole, quando per esempio siamo in presenza di uno sforzo, relativamente breve nel tempo, ma che impegna fortemente le riserve energetiche, e questo naturalmente tutti lo sappiamo per l'utilizzazione della caffeina nello sport.

Io credo perciò che la caffeina debba essere considerata come un farmaco non solo molto attivo — su questo siamo tutti d'accordo — ma molto selettivo, perciò un farmaco che deve essere meglio conosciuto anche nelle sue dosi. Non credo sia accettabile ciò che avviene oggi, cioè avere delle miscele di vari tipi di caffè o di varie forme di caffè, in cui la quantità di caffeina è ignota a chi la prende, credo che debba essere nota. Se noi ci avviamo verso un maggior controllo degli alimenti, anche dal punto di vista farmacologico, noi abbiamo il dovere e il diritto di sapere le dosi di questi farmaci potenti che noi ingeriamo, per il nostro bene e anche per evitare il nostro male, in un caso meno frequente, ma che può sempre esservi.

Vorrei ricordare a questo proposito che l'effetto della caffeina sul sistema nervoso è stato messo in luce nientemeno che da Morosini, per primo, che era il delegato della Repubblica Veneta a Cipro. In una sua lettera del 1570 già suggeriva ai Veneziani l'uso del **cavè**, nome turco usato ancora in Ungheria e in altri Paesi per il caffè, perchè, dice, « tiene gli uomini svegli », e lo somministrava ai suoi collaboratori. Evidentemente c'era già allora questo senso di stimolo artificiale sui collaboratori.

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DEPARTMENT OF PHYSIOLOGY, UNIVERSITY OF MILAN, ITALY

PLASMA LEVELS OF CAFFEINE AFTER ORAL, INTRAMUSCULAR AND INTRAVENOUS ADMINISTRATION

BY

G. SANT'AMBROGIO, P. MOGNONI AND L. VENTRELLA

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Considering the extensive therapeutic use of caffeine and its even wider ingestion through beverages like coffee, tea, etc... the absorption pattern of this drug after oral, intramuscular and intravenous administration should be profitably known in details.

AXELROD and REICHENTAL (1), comparing plasma levels of caffeine after oral and intravenous administration, reached the conclusion that this drug is completely and rapidly absorbed from the gastrointestinal tract. They also found that the rate of its metabolism is proportional to its concentration in blood, the average half reaction time being 3.5 hours. The same authors estimated that caffeine is distributed in various tissues in proportion to their water content.

We have studied the time course of the plasma levels of caffeine after oral (caffeine alkaloid), intramuscular and intravenous (caffeine-sodium benzoate) administration.

MATERIAL AND METHODS

Plasma caffeine has been estimated according to AXELROD and REICHENTAL (1).

The experiments have been performed on 5 male subjects to whom caffeine was administered either orally (in about 200 ml of water) or through an intramuscular injection. The intravenous administration was made on 3 male subjects and on 3 dogs. The dose ranged from 6 to 9 mg/kg body weight. Blood samples were withdrawn from the cubital vein in man and from the inferior vena cava in dogs, through a polyethylene cannula which was introduced into the femoral vein.

The blood samples were taken, mostly, at time intervals of 15, 30, 60, 90 and 150 min after the oral and intramuscular administration, and of 2, 5, 10, 20, 40 and 60 min after the intravenous injection.

Results and Discussion

Following oral administration peak plasma levels of caffeine were found to occur after 30 min in all five experiments (Fig. 1).

In the case of intramuscular injection the peak plasma levels of caffeine occurred constantly after about 60 min (Fig. 1).

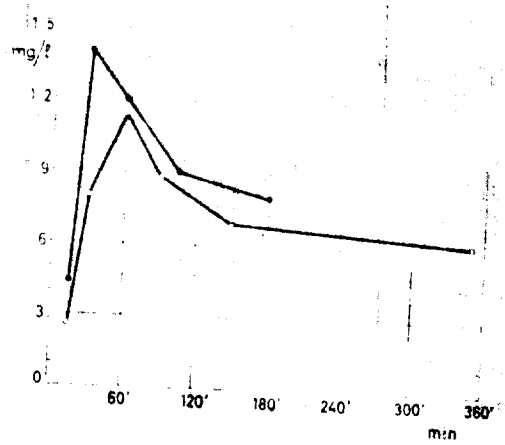

Fig. 1

Plasma levels of caffeine after oral (●) and intramuscular (○) administration; 0.5 g of caffeine was given in both the two experiments to the same subject weighing 80 kg.

It may seem surprising that the rate of absorption of caffeine is lower in the case of the intramuscular injection than after oral administration. This fact, which was constantly found in all our experiments, may be understood considering that caffeine, as prepared for i.m. injections, is in solution with sodium benzoate, which markedly increases its solubility: presumably the benzoate is absorbed into the blood stream very rapidly, and the caffeine for its poor solubility is then absorbed very slowly. On the other hand one should also take into account the great absorption capability of the gastrointestinal tract. The absorption from the muscle into the blood stream is dependent on the vascularization of the tissues which, in turn, can be affected, mechanically and or chemically, by the injection in a manner not always predictable.

It is interesting to know that the effect of caffeine, administered orally, on some respiratory parameters, reaches its maximum value after 30 min; i.e. at the time when its plasmal level has reached the peak value (2).

The plasma levels of caffeine, following intravenous injection, show a decrement which is especially rapid in the first minutes, due to the diffusion of the alkaloid from the blood into the tissues.

Since the caffeine is removed from the body only by metabolic processes, its renal excretion being negligible (1), it is possible to study analytically the time course of plasma levels of this drug considering only the processes of (a) diffusion from the blood into the tissues and of (b) metabolism. Either the diffusion (y_a) or the metabolic processes (y_b) have an exponential pattern, the first, being a fast one, with a very steep incline of the blood concentration curve, the second with a much less steep incline, the process being a much slower one. The plasma concentration of caffeine (y) should be accordingly a function of time (t), as described by the following equation:

$$y = y_a + y_b = C_1 e^{-at} + C_2 e^{-bt} \qquad (1)$$

where a and b are the velocity constants and C_1 and C_2 the concentration values at $t = 0$ for the diffusion, respectively the metabolic processes. C_1 is the concentration of caffeine if this were dissolved in a volume of liquid equal to the blood volume; C_2 is the concentration that caffeine would attain if dissolved uniformely through the total body water.

By plotting the experimental data on semi-logaritmic paper, a curve which represents the time course of caffeine plasma levels after intravenous injection is obtained, from which the rapid (diffusion) and the slow (metabolism) components can easily be evaluated (FIG. 2). For time values higher than 10 min the function is a straight line, which indicates that the diffusion process comes practically to an end before this interval: the function described by the straight line is, therefore, the expression of the metabolic process and it has been calculated as obeying the following equation:

$$\log y_b = 1.1635 - 0.0015\ t \qquad (2)$$

For time values below 10 minutes, by subtracting from the experimental data the values as given by equation (2), new data are obtained, which plotted on semi-logaritmic paper give another linear function, much steeper than the previous one, which is described by the equation:

$$\log y_a = \log(y - y_b) = 1.42 - 0.22\ t \qquad (3)$$

This function describes the diffusion process of caffeine from the blood into the tissues. The half reaction time of this process is about 1 min

and 30 sec i.e. the caffeine diffusion process through the vascular walls is very rapid, being of the same order of magnitude of that found for the lactic acid, a common metabolite, having a low molecular weight (3).

The rate of biotransformation of caffeine is such that the half reaction time of this process varies from 2 h and 20 min to 4 h and 30 min, with an average of 3 h and 5 min. These data are in agreement with those reported by AXELROD and REICHENTAL (1). This rate of biotransformation has been found to change, even in the same subject, in successive experiments, showing some degree of variability in the metabolic function of the tissues for the caffeine.

A practical conclusion of these experimental data is that the intramuscular injection of caffeine is not justified from the standpoint of absorption rapidity, unless there are reasons to suspect an impairment of the absorption process from the gastrointestinal tract.

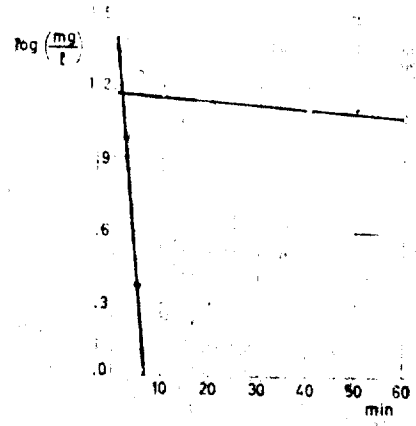

FIG. 2

Plasma levels of caffeine, log (mg/l), after intravenous injection of 0.13 g. Dog weighing 14 kg.

Obviously the very high plasma levels of caffeine found in the very first minutes after the intravenous injection are not related directly with the pharmacological action of the drug on the tissue elements, since this is dependent on the concentration of the substance in the tissue. This cannot be higher than that indicated by the C_2 component of equation (1), and this value is reached after about 10 min (FIG. 2), when a concentration equilibrium between blood and tissues is attained; from this moment on the blood concentration of caffeine is representative of the tissue concentration and should also be related to the biological [illegible] of this alkaloid.

Summary

1) The plasma levels of caffeine after oral, intramuscular and intravenous administration have been measured.

2) After oral administration peak plasma levels of caffeine were reached in 30 min.

3) The absorption following intramuscular injection was consistently found to be slower, i.e. the peak plasma levels of caffeine were reached in 60 min.

4) The plasma levels of caffeine, following intravenous injection, are susceptible of a quantitative evaluation: they decrease rapidly in the first minutes, as the alkaloid diffuses from the blood into the tissues. The half reaction time of this diffusion process is about 1 min and 30 sec.

5) The rate of metabolism of caffeine has an average half reaction time of 3 h and 5 min.

References

1. — Axelrod, J. and Reichental, J. *J. Pharmacol.*, 1953, *107*, 519.
2. — Sant'Ambrogio, G., Mognoni, P. Torri, G., and Agostoni, E. *Rend. Accad. Naz. Lincei*, 1963, *34*, 314.
3. — Milla, E. and Capraro, V. *Boll. Soc. Ital. Biol. Sper.*, 1940, *15*, 340.

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HEART DISEASE DUE TO ACUTE CAFFEINE POISONING

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In the literature available to us, we found no information on acute intoxication by caffeine associated with heart disease. A. P. Khodasevich described two actual cases of acute caffeine poisoning; one of the sufferers died. Clinical, electrocardiograph, and other instrumental and laboratory data on the heart condition were not presented by the author. We present our observations.

A patient, T., 40 years of age, a technician, entered the clinic on May 30, 1968. He had worked for eight years on the synthesis of sodium benzoate. In the course of a week's time, he went down into the drying room several times a day (for 15-20 minutes), where there was a large amount of powdered caffeine suspended in the air and on the floor. After work, he noted palpitation of the heart, which occurred for 30-40 minutes in the fresh air. In the night between the 29th and 30th of May, 1968, he cleaned a chamber with a shovel (using no respirator), where there were approximately 500 kg of sodium benzoate of caffeine. 1-1½ hours after beginning work, a feeling of shortness of breath, fever, and general weakness appeared. Leaving the chamber, he busied himself with other work. After two-three hours, he felt poorly, with heart palpitation, slight head-spinning, nausea, and body chills. Before the end of the shift, he was dismissed to go home. On the way, his shortness of breath increased, nausea and a single instance of vomiting occurred, and at home, he had a feeling of pulsation in the temples, cramps in the lower extremities, and a weakening in general condition. First aid (stomach irrigation) was rendered by a doctor in emergency, after which the patient entered the clinic.

Upon admission, he complained of frequent stabbing in the region of the heart, heart palpitation, chills in the whole body, and cramps in the abdominal muscles, forearms, and calf muscles. There were no sorts of indications of previous disease or industrial poisoning. The patient used alcohol in moderate amount, taken occasionally. His mind was clear. The skin of the face and chest were sharply hyperemic. His speech was rapid and somewhat blurred. Chill-like shaking of the body, general agitation, motor disturbance, and euphoria were present. The patient appeared older than his years. Shortness of breath, respiration in stiffened lungs, and breathing rate at 26 times per minute were observed. The pulse was 110 per minute, tense and rhythmic. The peaks were spread out, the heart boundary was displaced to the left 1 cm, and tonus was increased. Arterial pressure was 160-80 mm. The abdomen was soft;

palpitation defined a not-so-sharp pain in the muscles of the upper half of the abdomen. In a study of basal reflexes, there were tonic cramps of the calf muscles.

On the EKG, recorded on the day of admission (see fig. a), signs were noted of subepicaridal ischemia of the myocardium, primarily of the top and side portions of the rear wall of the left ventricle. Sinus tachycardia was 110-115 systoles per minute. The spur T_1 is crooked, the spur T is parts II, III, and V_{5-6} in the lower parts are two-phased, and the spur T in parts V_{3-4} is two-peaked.

Magnesium bisulfate was administered to the patient intramuscularly, as well as aminazine with novocaine, and hot water bottles were applied to the legs. Two hours after awakening, the patient noted some improvement in how he felt. There remained a marked hyperemia of the face, chills in the body, and hand tremors. Frequency of respiration was 20 times per minute, pulse 113 per minute. Arterial pressure was 140-80 mm. A short-term, repetitive cramp was observed in the calf muscles.

Fig. 1. EKG of the patient, T., 40 years of age, on the day of admission (a), on the 2nd day (b), on the 12th day (c), and after three months (d) after caffeine poisoning.

After receiving chloral hydrate, the sufferer fell asleep. In the morning, the patient was somewhat agitated, talkative, excessively active, and euphoric. The skin of the body, especially the face, neck, back, and chest, was significantly hyperemic, dry, and warm. Morbidity was determined by palpitation of the calf muscles. Pulse was somewhat decreased (102 per minute) and diastolic arterial pressure was slightly increased (150/95 mm). There was very slight hyperaesthesia of the feet. Dermographism was white and unstable.

On an EKG recorded on May 31, 1968 (see fig. b), changes began to be more marked. Tachycardia increased (to 150 per minute). The S-T segment decreased in parts II, III, and V_{4-6}; spur T in parts II, III, and V_{4-6} became distinctly two-phased (with a large negative phase).

With X-ray observation of the chest cavity on May 3, 1969, the heart was extended horizontally and the left ventricle was somewhat enlarged. Fibrocaseous tuberculosis of the right lung was present. An analysis of blood and urine was normal.

In the course of the following days, the patient's condition improved noticeably. Respiration rate (16 per minute), arterial pressure (130/80 mm), and pulse (80 per minute) were normalized. Excess activity and fussiness, with elements of euphoria, were observed. In the subsequent days, the phenomena enumerated continuously smoothed out; however, shortness of breath under physical load and rapid pacing were still noted. The EKG (from June 12, 1968), taken in the 12th day of illness

(as fig. c) still indicated small changes in the myocardium (a lowering of T in parts II and V_{4-6}).

On the 7th to 10th days of the patient's stay at the clinic, a two-fold increase was noted in glutamine-aspargine transaminase, to 82 units (for a norm of 40 units), as well as an insignificant increase in excretion of noradrenaline in urine - over 24 hours, to 24 ug (for a norm of 20 ug).

The clinical diagnosis was acute occupational caffeine poisoning, accompanied by myocardial disease.

The patient signed out on the 13th day in satisfactory condition. In a check-up three months later, there was no aberration on the part of the internal organs or the nervous system. The EKG was normal (see fig. d).

The changes observed in the patient on the part of the nervous and cardiovascular systems, in agreement with the data of Sel'ye, P. D. Gorizontov, and Raab, may be explained as the occurrence of a complex of protective adaptation reactions by the organism to a type of "stress". Because of this, a large amount of catecholamine is noted entering the blood; it is excess supply in the heart muscle, and hypoxia of the myocardium may occur, as well as necrosis, most often localized in the subendocardial and subepicardial layers of the myocardium and pallar muscles. There are data (V. N. Burdina and Ye. F. Melekhova) which indicate that large doses of caffeine activate the hypophyseal-adrenal core system. After administering large doses of caffeine to rabbits, significant phase oscillations are observed in the levels of adrenalin and adrenalin-like substances in the blood, as well as a distinct increase in adrenalin content in the adrenal glands (M. R. Ozerova). Tachycardia is noted in the animals, along with polytopic extrasystole, ventricle thrythm, and other EKG changes. V. Ya. Gorodinskaya associated these disruptions with over excitation of the adrenergic system in the myocardium, in which, because of this, an increase was observed in the activity of phosphorilizing processes and a decrease in glycogen content (A. I. Cherkes and N. M. Dmitriyeva).

The results of the study, as well as the data in the literature, permit the assumption that the stress state observed in the patient, and occurring as a result of caffeine action, led to the disruption of metabolism in the heart, the occurrence of hypoxia, distrophy of the myocardium, and possibly low-threshold necrosis.

Heart changes in the patient with acute caffeine poisoning are nonspecific, as our observations and the data of a number of authors indicate; these data describe similar changes in the heart under the influence of other substances (A. S. Savina, A. O. Saytanov, N. Z. Lazarev, and others).

Literature Cited

Burdina, V. N., and Melekhova, Ye. F., Abstracts from the Scientific Conf. on the Action of Pharmacological Substances on the Endocrine Glands, Leningrad, p. 13 (1965).

Cherkes, A. I., and Dmitriyeva, N. M., in: Pharmacology and Chemistry, Moscow, p. 381 (1965).

Gorizontov, P. D., Abstracts from the 3rd All-Union Conf. of Pathophysiologists, Moscow, p. 49 (1960).

Gorodinskaya, V. Ya., in: Pharmacology and Chemistry, op. cit., p. 95.

Khodasevich, A. P., Pharmacology and Toxicology, p. 62 (1957).

Lazarev, N. V., Harmful Substances in Industry, Leningrad (1954).

Ozerov, M. R., Problems of Endocrinology, No. 4, p. 54 (1955).

Raab, W., Dis. Chest., 53, p. 629 (1968).

Savina, A. S., in: Proc. 1st All-Union Scientific Practitioners' Conf. on Diagnostics, the Clinic, and Curing Acute Poisoning, Moscow, p. 47 (1968).

Saytanov, A. O., Clin. Med., No. 11, p. 60 (1966).

Sel'ye, G., Reviews on Adaptation Syndrome, Moscow, p. 203 (1960).

Vincent, D., and Porant, M., C. R. Soc. Biol., 148, p. 1078 (1954).

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ПОРАЖЕНИЕ СЕРДЦА ПРИ ОСТРОМ ОТРАВЛЕНИИ КОФЕИНОМ

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В доступной нам литературе мы не встретили сообщений об острой интоксикации кофеином, сопровождающейся поражением сердца. А. П. Ходасевич описал 2 случая острого бытового отравления кофеином: один из пострадавших умер. Клинические, электрокардиографические и другие инструментальные и лабораторные данные о состоянии сердца автором не были представлены. Приводим наше наблюдение.

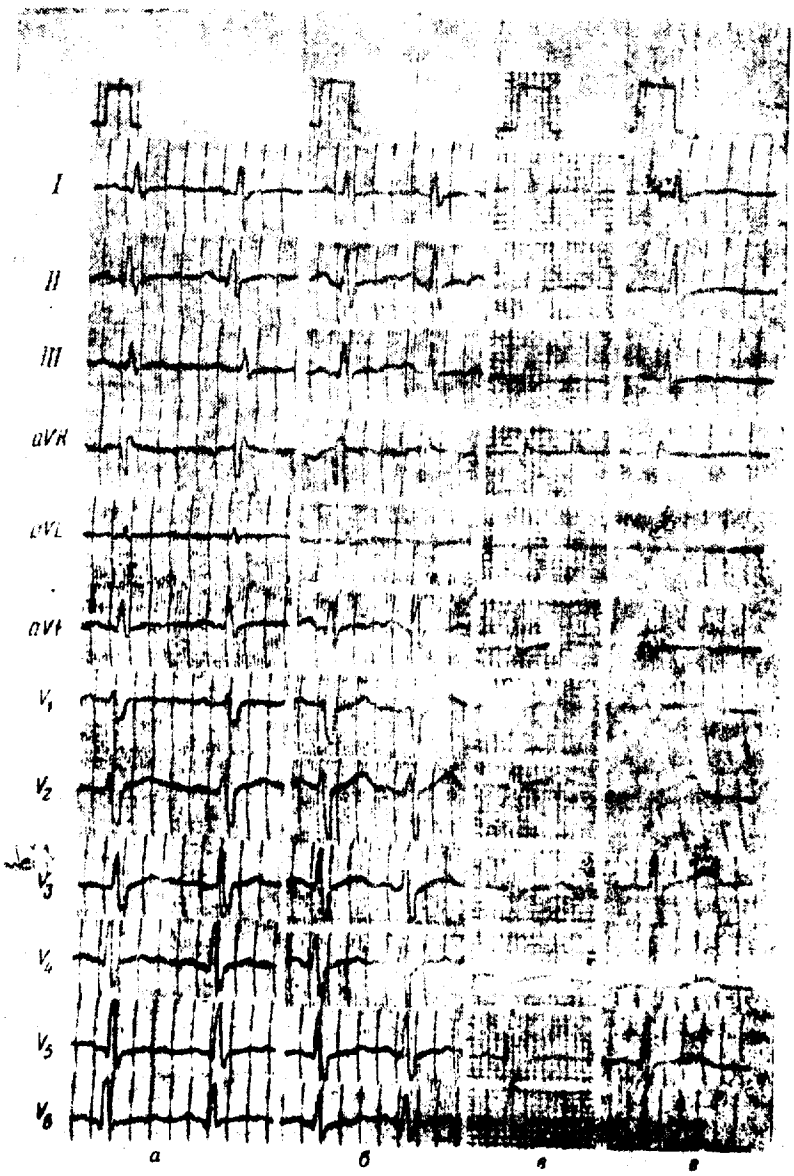
Больной Т., 40 лет, аппаратчик, доставлен в клинику 30/V 1968 г. Работает (8 лет) на синтезе бензоната натрия. В течение последней недели за время работы приходилось несколько раз в день спускаться (на 20—15 мин.) в сушильную камеру, где во взвешенном состоянии в воздухе, а также на полу содержалось большое количество порошка кофеина. После работы отмечал сердцебиение, проходившее на воздухе через 30—40 мин. В ночь с 29 на 30/V 1968 г. чистил лопатой (без респиратора) камеру, где находилось приблизительно 500 *кг* бензойно-натриевой соли кофеина. Через 1—1½ часа после начала работы появились ощущение недостатка воздуха, жар и общая слабость. Выйдя из камеры, занялся другой работой. Через 2—3 часа почувствовал недомогание, сердцебиение, легкое головокружение, тошноту и дрожь в теле. До окончания смены был [illegible] По дороге усилилась одышка, появилась тошнота и однократная рвота, [illegible] пульсации в висках, судороги нижних конечностей, ухудшение общего [illegible] Врачом неотложной помощи была оказана первая помощь (промывание желудка), [illegible] больной доставлен в клинику.

При поступлении предъявлял жалобы [illegible] сердцебиение, дрожь во всем теле, судороги [illegible] живота, предплечий и икр [illegible] В анамнезе нет каких-либо указаний [illegible] сопутствующие заболевания и [illegible] интоксикации. Алкоголь употребляет и [illegible] в умеренных дозах. [illegible] лица и груди резко гиперемирована. Речь быстрая, несколько смазанная. [illegible] кожание тела, общее возбуждение, [illegible] беспокойство, [illegible] выглядит старше своих лет. Одышка, дыхание в легких жесткое, [illegible]

26 в минуту. Пульс 110 в минуту, напряженный, ритмичный. Верхушечный толчок разлитой, граница сердца смещена влево на 1 см, тоны усилены. Артериальное давление 150/80 мм. Живот мягкий, при пальпации определяется резкая болезненность в мышцах верхней половины живота. При исследовании подошвенных рефлексов тонические судороги икроножных мышц.

На ЭКГ, зарегистрированной в день поступления (см. рисунок, *а*), отмечались признаки субэпикардиальной и субэндокардиальной ишемии миокарда, преимущественно верхушечно-бокового отдела и задней стенки левого желудочка сердца. Синусовая тахикардия — 110—115 сокращений в минуту. Зубец T_1 сглажен, зубец T в отведениях II, III, V_{5-6} низкий двухфазный, зубец T в отведениях V_{3-4} двугорбый.

Больному введены внутримышечно сернокислая магнезия, аминазин с новокаином и положены грелки к ногам. Через 2 часа после пробуждения больной отмечал некоторое улучшение самочувствия. Оставались выраженная гиперемия лица, дрожание тела и тремор рук. Частота дыхания 20 в минуту, пульс 113 в минуту. Артериальное давление 140/80 мм. Наблюдались кратковременные повторные судороги в икроножных мышцах.



ЭКГ больного Т., 40 лет., в день поступления (*а*), на 2-й (*б*), на 12-й день (*в*) и через 3 месяца (*г*) после отравления кофеином.

После приема хлоралгидрата пострадавший снова уснул. Утром больной несколько возбужден, говорлив, излишне подвижен, эйфоричен. Кожа тела, особенно лица, шеи, спины и груди значительно гиперемирована, сухая и теплая. Определялась болезненность при пальпации икроножных мышц. Несколько замедлился пульс (102 в минуту) и слегка повысилось диастолическое артериальное давление (150/95 мм). [illegible] тезия стоп. Дермографизм белый, нестойкий.

На ЭКГ, зарегистрированной 31/V 1968 г. (см. рисунок, [illegible] выраженными. Усилилась тахикардия (до 150 в минуту). [illegible] отведениях II, III, V_{4-6}, зубец T в отведениях II, III, V_{4-6} [illegible] ным (с большей отрицательной фазой).

При рентгеноскопии грудной клетки 3/V 1969 г. сердце расположено горизонтально, нерезко увеличен левый желудочек. Пульсация возбужденная, малой амплитуды. Аорта слегка развернута. Обнаружен фиброзно-очаговый туберкулез правого легкого. Анализы крови и мочи нормальные.

В течение последующих дней состояние заметно улучшилось. Нормализовано число дыханий (16 в минуту), артериальное давление 130/80 *мм*, нормализовано [illegible] (80 в минуту). Наблюдались еще излишняя подвижность, суетливость, элементы эйфории. В последующие дни перечисленные явления постепенно сгладились, однако еще отмечалась одышка при физической нагрузке и быстрой ходьбе. ЭКГ (от 12/VI 1968 г.), снятая на 13-е сутки заболевания (см. рисунок, *в*), еще указывала на небольшие изменения миокарда (снижение T в отведениях II, V_{4-6}).

На 7—10-й день пребывания больного в клинике было обнаружено повышение почти в 2 раза глютамино-аспарагиновой трансаминазы крови до 82 ед. (при норме до 40 ед.) и незначительное увеличение экскреции норадреналина с мочой — за сутки до 24 *мкг* (при норме до 20 *мкг*).

Клинический диагноз: острое производственное отравление кофеином, сопровождающееся поражением миокарда.

Больной выписан на 13-е сутки в удовлетворительном состоянии. При осмотре через 3 месяца отклонений со стороны внутренних органов и нервной системы не обнаружено. ЭКГ нормальная (см. рисунок, *г*).

Изменения, наблюдавшиеся у больного со стороны нервной и сердечно-сосудистой систем, согласно данным Селье, П. Д. Горизонтова, Raab, можно попытаться объяснить возникновением сложного комплекса защитной адаптационной реакции организма типа «стресса». При этом отмечается большое поступление катехоламинов в кровь, их избыточное накопление в сердечной мышце и могут возникнуть гипоксия миокарда, а также некрозы, чаще локализованные в субэндокардиальных и субэпикардиальных слоях миокарда и папиллярных мышцах. Имеются данные (В. Н. Бурдина и Е. Ф. Мелехова), свидетельствующие, что большие дозы кофеина активизируют систему гипофиз — кора надпочечников. После введения кроликам больших доз кофеина наблюдаются значительные фазные колебания уровня адреналина и адреналиноподобных веществ в крови и отчетливое увеличение содержания адреналина в надпочечниках (М. Р. Озерова). У животных отмечаются тахикардия, политопная экстрасистолия, желудочковый ритм и другие изменения ЭКГ. Эти нарушения В. Я. Городинская связывает с перевозбуждением адренергической системы в миокарде, в котором при этом обнаруживается увеличение активности процессов фосфорилирования и снижение содержания гликогена (А. И. Черкес и Н. М. Дмитриева).

Результаты исследования, а также данные литературы позволяют предположить, что наблюдавшееся у больного стрессовое состояние, возникшее в результате действия кофеина, привело к нарушению метаболизма в сердце, возникновению гипоксии, дистрофии миокарда и, возможно, к мелкоочаговому некрозу.

Изменения сердца у больного с острым отравлением кофеином не являются специфичными, о чем свидетельствуют наши наблюдения и данные ряда авторов, описавших аналогичные изменения со стороны сердца при действии других веществ (А. С. Савина; А. О. Сайтанов; Н. В. Лазарев, и др.).

ЛИТЕРАТУРА

Бурдина В. Н., Мелехова Е. Ф. Тезисы докл. научной конференции. Действие фармакологических веществ на эндокринные железы. Л., 1965, с. 13. — Горизонтов П. Д. Тезисы докл. 3-й Всесоюзн. конференции патофизиологов. М., 1960, с. 49. — Городинская В. Я. В кн.: Фармакология и химия. М., 1965, с. 95. Лазарев Н. В. Вредные вещества в промышленности. Л., 1954. — Озерова М. Р. Пробл. эндокринол., 1955, № 4, с. 54. — Сайтанов А. О. Клин. мед., 1966, № 11, с. 60. — Савина А. С. В кн.: Материалы 1-й Всероссийск. научно-практической конференции на тему: диагностика, клиника и лечение острых отравлений. М., 1968, с. 47. — Ходасевич А. П. Фармакол. и токсикол., 1957, прилож. к ж. за 1956 г., с. 62. — Черкес А. И., Дмитриева Н. М. В кн.: Фармакология и химия. М., 1965, с. 381. — Raab W., Dis. Chest, 1968, v. 53, p. 629. — Селье Г. Очерки об адаптационном синдроме. М., 1960, с. 203. — Vincent D., Poirant M., C. R. Soc. Biol., 1954, v. 148, p. 1078.

AFFECTION OF THE HEART IN ACUTE CAFFEINE POISONING

A. O. Sa.tanov

Summary

The paper presents a detailed clinico-instrumental description of a 40-year-old man with acute inhalation poisoning with powdered caffeine. The clinical picture showed distinct signs of excitation of the adrenergic system this, apparently, being the cause of ECG and EMG changes of muscles of the extremeties, testifying to metabolic disorders. In view of the dynamics of ECG changes, the augmented level of asparaginic (muscle) transaminase of the blood the author assumes the presence of myocardial dystrophy and microfocal necrose in the myocardium and skeletal musculature In contrast to the known acute poisoning with caffeine this case was characterized by a somewhat delayed picture of intensification and reverse development of symptoms of acute caffeine poisoning associated with the inhalation route of intoxication.

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UNUSUAL CONSEQUENCES OF CAFFEINE CONSUMPTION

by

Dieter Schulte (Rostock)

Caffeine is a favored agent for use as an analeptic. Besides centrally determined circulation-toning effects, like most purine materials, it possesses spasmolytic properties, which affect especially the vessels of the cerebral cortex. Although unpleasant side effects are considered to be extremely slight, too great and especially an uncustomary consumption of coffee can lead to quite considerable disorders, as the personal observation we will describe and the experience of another physician reveal:

On two successive holidays, a completely uncustomarily large amount of bean coffee was consumed: on the first day, 3 cups in the afternoon, an additional 2 to 3 cups of tea in the evening, then 6-8 cups on the following day, and in the evening, several cups of tea. The night's sleep was nonetheless good and sufficient. The next morning, on the third day, 2 more cups of very strong coffee were drunk. About 8 hours later, after a rather lengthy train ride, though the subject felt well otherwise, entoptic scotomas appeared, and led immediately to a left-side hemianopsia. This latter was not directly apparent, but was discovered soon after, when the subject was reading. After about a half hour,

the vision disorders had disappeared. Simultaneously there began headaches, which became even more intense during a walk in the course of the next hours. After a deep night's sleep, all the symptoms had disappeared by the next morning.

Another observation from the Rostock clinic concerns a 32 year-old physician, who had never previously complained of vision disorders, but who one day, two to three hours after a good deal of afternoon coffee (several cups of very strong black coffee), could not see out of either eye. Amid simultaneous entoptic phenomena, the vision in both eyes went black. He took some nitrolingual, and within the next few hours was able to see somewhat again. However, intense and long-lasting headaches soon appeared. Not until the next morning did all the disturbances disappear.

At least in the first case we can speak of an excess of caffeine, since for several successive days, several times the therapeutic dose (there about 0.1 grams of caffeine in one cup of bean coffee) had been consumed.

How can we explain the consequent vision disorders? According to our views, the entoptic scotoma with hemianopic symptoms is due to spasms of the brain vessels. Although caffeine stimulates the vasomotory center, thus effecting a general vasal constriction through central stimulation, the effect of a vasodilatation directly attacking the peripheral vasal apparatus is exercised on the brain, as well as on enervated organs (Meyer-Gottlieb: Experimental Pharmacology). However, a contrary post-effect must also be possible, as the two observations demonstrate. Either the long-lasting stimulation of the vaso-dilatory apparatus leads to extensive fatigue of the same, so that after the caffeine effect has worn off

opposite regulations take over, making possible a spasm in the brain area, or, and this explanation is the more plausible, the centrally released vasoconstricting effect lasts longer than the peripheral vaso-dilatory one, at least after stronger and possible protracted doses. Since caffeine, especially after larger doses, can manifest certain brain-paralyzing phenomena, in the form of mental distraction, it is also possible that a pharmacologically determined secondary dulling of the vision cortex can be the cause of the vision disorders. It is true that this explanation, which is not based on vasal effects, has little support, since the headaches typical of both cases certainly must be related to a migraine-like vasal condition. Whichever assumption may be true, the fact that after the consumption of a substance that is also used extensively as a medication, we observe the precise condition that otherwise is supposed to be affected therapeutically by this substance, is thoroughly unusual and deserves a certain attention. Obviously the "paradoxical" caffeine effect described is to be viewed as the consequence of a relative overdosage, which naturally corresponds to different amounts according to the individual and his condition of habituation to the substance.

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geben sich keine Anhaltspunkte für die Keratoconusnatur. Am rechten Auge findet sich zwar eine Pigmentlinie, beiderseits sind aber keine Keratoconusspaltlinien festzustellen, trotzdem der Patient bereits das 56. Lebensjahr erreicht hat. Die geringe Verdünnung der Hornhaut ist nicht für Keratoconus beweisend. Wie *Vogt* betont, ist der Astigmatismus rectus bei Keratoconus so häufig zu finden, daß ihm eine gesetzmäßige Ursache zugrunde liegen muß. Auch davon weicht meine Beobachtung ab, da es sich um einen inversen Astigmatismus handelt.

Ob bei meinem Fall die Hornhautanomalie erblich bedingt ist, läßt sich nicht feststellen. Welches Augenleiden bei der Tante mütterlicherseits vorlag, konnte leider nicht in Erfahrung gebracht werden. Da für den Hornhautastigmatismus erbliche Momente anzunehmen sind, müssen diese wohl auch für den Keratotorus vermutet werden. Die torische Verwölbung der Hornhaut wirkt sich offenbar auch auf die Gesamtgestaltung des Augapfels aus, wie dies die myopischen Augenhintergrundsveränderungen meines Falles zeigen. Zwischen den cornealen und skleralen Ektasien scheinen nähere Beziehungen zu bestehen, *Kraupa* hat auf das auffallende Vorkommen des Keratoconus in Familien mit erblicher hochgradiger Myopie hingewiesen. *Franceschetti* vermutet beim Keratoconus ein digen dominantes Leiden. *Van der Hoeve* teilte einen Stammbaum einer Familie mit, in dem neben Keratoconus auch Astigmatismus häufig vorkommt. *Franceschetti* meint, daß Individuen mit nur einem krankhaften Gen leichtere Störungen der Hornhautkrümmung und -dicke zeigen könnten. Ob es sich bei meinem Keratotorus um ein Individuum aus einer Familie mit einem derartigen Gen handelt, läßt sich nicht feststellen. Jedenfalls möchte ich durch meine Ausführungen zu weiteren Untersuchungen in dieser Richtung anregen. ***Solange keine sicheren Beziehungen zwischen Keratoconus und Keratotorus festgestellt sind, ist es berechtigt, diesen als eigene Anomalie anzuerkennen.***

XXVIII.

Herr *Dieter Schulte* (Rostock): *Ungewöhnliche Folgen nach Coffeingenuß.*

Das Coffein ist als Analeptikum ein beliebtes Genußmittel. Neben zentral bedingten kreislauftonisierenden Wirkungen besitzt es wie die meisten Purinstoffe spasmolytische Eigenschaften, die besonders an den Gefäßen der Hirnrinde zur Geltung kommen. Obwohl unangenehme Nebenwirkungen als äußerst geringfügig angesehen werden, vermag ein zu starker und vor allem ungewohnter Kaffeegenuß zu nicht unerheblichen Störungen zu führen, wie eine zu schildernde Selbstbeobachtung und das Erlebnis eines anderen Arztes erweisen sollen:

An zwei aufeinanderfolgenden Festtagen wurde eine völlig ungewohnte größere Menge Bohnenkaffees genossen: Am ersten Tag nachmittags 3 Tassen, abends zusätzlich 2—3 Tassen Tee, über den nächsten Tag verteilt 6—8 Tassen, abends wiederum mehrere Tassen Tee. Trotzdem jedesmal guter und ausreichender Nachtschlaf. Am nächsten Morgen, also am 3. Tag, wurden wie

derum 2 Tassen stärksten Bohnenkaffees getrunken. Ungefähr 8 Stunden später setzten nach längerer Bahnfahrt bei sonst völligem Wohlbefinden Flimmerskotome ein, die sofort zu einer linksseitigen Hemianopsie führten. Letztere trat nicht ohne weiteres ins Bewußtsein, sondern wurde gleich hinterher beim Lesen entdeckt. Etwa nach einer halben Stunde waren die Sehstörungen behoben. Gleichzeitig begannen Kopfschmerzen, die sich während eines Spazierganges im Laufe der nächsten Stunden noch verstärkten. Nach einem tiefen Nachtschlaf waren am anderen Morgen alle Erscheinungen geschwunden.

Eine weitere Beobachtung aus der Rostocker Klinik betrifft einen 32jährigen Arzt, der niemals vorher über Sehstörungen zu klagen hatte, aber eines Tages 2—3 Stunden nach ausgedehntem Nachmittagskaffee (mehrere Tassen sehr starken Bohnenkaffees) auf beiden Augen plötzlich nichts mehr sehen konnte. Es sei unter gleichzeitigen Flimmererscheinungen vor beiden Augen dunkel geworden. Er nahm Nitrolingual und konnte im Verlauf der nächsten Stunden wieder etwas sehen. Es traten aber bald heftige und langanhaltende Kopfschmerzen auf. Erst am anderen Morgen waren alle Beschwerden gewichen.

Mindestens im ersten Fall kann man von einem Coffeinexzeß sprechen, da an mehreren Tagen hintereinander das mehrfache der therapeutischen Dosis (in einer Tasse Bohnenkaffee sind durchschnittlich etwa 0,1 Gramm Coffein enthalten) eingenommen wurde.

Wie sind die nachfolgenden Sehstörungen zu erklären? Nach unseren Anschauungen beruht das Flimmerskotom mit hemianopischen Erscheinungen auf Spasmen der Hirngefäße. Obwohl Coffein das Vasomotorenzentrum anregt, also durch zentrale Reizung eine allgemeine Gefäßverengung bewirkt, setzt sich am Gehirn, wie übrigens auch an entnervten Organen, der Einfluß einer direkt am peripheren Gefäßapparat angreifenden Vasodilatation durch (*Meyer-Gottlieb:* Experimentelle Pharmokologie). Es muß aber eine konträre Nachwirkung möglich sein, wie die beiden Beobachtungen zeigen. Entweder führt die langanhaltende Reizung des gefäßerweiternden Apparates zur weitgehenden Ermüdung desselben, so daß nach Abklingen der Coffeinwirkung entgegengesetzte Regulationen das Übergewicht bekommen, und so ein Spasmus im Hirngebiet möglich ist, oder aber, und diese Erklärung hat am meisten für sich, die zentral ausgelöste vasokonstriktorische Wirkung überdauert die periphere vasodilatatorische, wenigstens nach stärkeren und eventuell protrahierten Dosen. Da das Coffein, besonders nach höheren Gaben, doch gewisse Hirnlähmungserscheinungen in Form von Verwirrtheit erkennen lassen soll, wäre auch eine pharmokologisch bedingte sekundäre Dämpfung der Sehrinde als Ursache für die Sehstörungen in Erwägung zu ziehen. Doch hat diese dritte nicht auf Gefäßwirkungen beruhende Erklärungsmöglichkeit von vornherein wenig für sich, da die in beiden Fällen typischen Kopfschmerzen sicher auf einen migräneartigen Gefäßzustand bezogen werden müssen. Welche Annahme auch zutreffen mag, daß wir nach einem Genuß-

[illegible] Vorbereitung gefunden hat, gerade

Zustand ausgelöst sehen, der sonst mit seiner Hilfe therapeutisch beein- werden soll, ist durchaus ungewöhnlich und verdient auch eine gewisse htung. Offenbar ist die geschilderte „paradoxe" Coffeinwirkung als e einer relativen Überdosierung anzusehen, die natürlich individuell und je nach dem Zustande der Gewöhnung durchaus verschiedenen Quanten bricht.

XXIX.

Dieter Schulte (Rostock): *Eigenartige Komplikationen der Dakryophlegmone am Augapfel.*

Die Komplikationsmöglichkeiten der Dakryophlegmone sind vielseitig. gefürchtesten dürfte eine Orbitalphlegmone sein. Teils wird eine Fort- ıg der Noxe auf dem Lymphwege, teils über eine Thrombophlebitis nommen, abgesehen von den direkt fortgeleiteten Entzündungen beim egen einer Nebenhöhlenerkrankung. Nach *Birch-Hirschfeld* (Kurzes lbuch d. Ophth. 3. S. 40) muß es vielfach offenbleiben, ob diese Orbital- dikation auf direktem fortgeleitetem Wege oder metastatisch entstan- st.

Noch viel seltener scheint eine Dakryophlegmone den Bulbus zu affi- ı, wobei natürlich nicht der exogenen Hornhautulceration gedacht soll.

Kürzlich konnten wir bei zwei Patientinnen der Rostocker Klinik eine itis im Gefolge einer Dakryophlegmone beobachten, ein Ereignis, das isher nicht beschrieben fanden.

Bei der ersten Patientin handelte es sich um eine 51 Jahre alte Frau la R.), die seit 5 Jahren über linksseitiges Tränen klagte. Bei der Auf- e bestand 4 Tage lang eine Schwellung mit Schmerzen in der Gegend nken Auges. Es fand sich eine typische Dakryophlegmone ohne Tem- ırsteigerung. Nach langsamem Rückgang der Krankheitserscheinungen ı 12 Tage nach Beginn wiederum starke Schmerzen auf, jetzt aber am ıfel der erkrankten Seite mit diffuser ziegelroter Verfärbung und mäßiger ellung der Sklera ohne konjunktivale Beteiligung. Keine Bewegungs- derung, keine Protrusio, die Iris war hyperämisch, die Vorderkammer Tension weich. Nach 14 Tagen konservativer Therapie erfolgte Heilung, darauf wurde mit gutem Erfolg eine Dakryozystorhinostomie durch- t.

ei der zweiten 63 Jahre alten Patientin (Regina Sch.) bestanden seit chen heftige Schmerzen in der Gegend des rechten Auges und Tränen- Es handelte sich um eine offenbar im Abklingen begriffene Dakryo- is phlegmonosa. Hier war bei der ersten Untersuchung schon eine diffuse itis mit iritischer Reizung vorhanden. Spätere Saccusexstirpation brachte ng auch der Skleritis.

Bei beiden Patientinnen war es kurz nach Ausbruch einer heftigen nsackphlegmone zur Skleritis auf dem Auge der erkrankten Seite nmen. Während einmal der [illegible]

konnte, und die Augenkomplikation am 12. Krankheitstag einsetzte, zeigte die zweite Patientin bei abklingender Tränensackphlegmone einen auf dem Höhepunkt stehenden skleritischen Entzündungszustand, der auch bald ohne wesentliche Behandlung abheilte. Bemerkenswert ist bei beiden Fällen die diffuse Iritis. Wir wissen ja, daß nur selten die Sklera allein erkrankt bleibt, eine Entzündung der Lederhaut ist fast immer mit einer Uveitis oder Keratitis verbunden. Am häufigsten entsteht sie überhaupt sekundär bei einer Gefäßhautentzündung. Hier scheint aber dieselbe Noxe gleichzeitig die Entzündung der Lederhaut wie der Regenbogenhaut ausgelöst zu haben.

Wie hat man sich die Pathogenese dieser Komplikation vorzustellen?

Nach *Eisler* (Kurzes Handbuch I, S. 38) besitzt die Sklera keine Lymphgefäße, so daß eine Fortleitung der Entzündung auf dem Lymphwege ausscheiden muß. Außer einer Fortleitung per continuitatem, die in unseren Fällen ausgeschlossen erscheint, ist nur eine Einschwemmung entzündlicher Noxen auf dem Blutwege möglich, entweder metastatisch, also infolge Übertragung durch die arterielle Strombahn, oder auf venösem Wege durch eine Thrombophlebitis. Eine arterielle Aussaat vom Tränensack in die Sklera ist allerdings unwahrscheinlich, denn dazu wird ein Einbruch infektiösen Materials in die Gesamtblutbahn Voraussetzung sein müssen. Die Skleritis bei septischen Erscheinungen zeigt sich aber ganz anders. Es kommt zur Episkleritis furunkuliformis oder zum Skleralabsceß, also zur umschriebenen eitrigen Lederhautentzündung, nicht zur diffusen Skleritis wie in unseren Fällen. Um so eher ist die Ausbreitung auf dem Venenwege denkbar. Bei einer Mitbeteiligung der Orbita würden wir diesen Ausbreitungsmodus geradezu fordern und uns nicht darüber wundern. Die Skleritis aber als unmittelbare Komplikation der Dakryophlegmone ist schwieriger zu erklären. Man muß dazu wohl direkte Anastomosen von Tränensackvenen mit Skleralvenen annehmen, so daß dann eine Thrombophlebitis von den Tränensackvenen ausgehend auf diese und die vorderen Ciliarvenen beschränkt bleiben kann. Nach länger zurückliegenden Untersuchungen von *Gurwitsch* (v. Gräfes Arch. Oph. 29, 4, S. 31) münden die Tränensackvenen in vereinzelten Fällen (zweimal bei 42 Untersuchten) nicht in die Vena angularis, sondern in die Vena ophthalmica sup., und nach *Krauß* (34. Vers. Dtsch. Ophthalm. Ges. Heidelberg 1907, S. 88) darf man sogar eine regelmäßige Kommunikation zwischen Tränensackvenen und einem venösen Ringsystem am vorderen Bulbus annehmen. Anastomosen zwischen Ciliarvenen und Tränensackvenen sind also möglich. Als Varianten würden sie zwanglos die Seltenheit der beschriebenen Komplikation erklären.

Nebenbei soll erwähnt sein, daß eine Skleritis außer bei Tränensackentzündung auch als Folge eines Erysipels zustande kommen kann, wie von uns kürzlich beobachtet wurde. Rechtsseitiges Gesichtserysipel bei einem ulcerierenden Basaliom des Nasenflügels führte zur Skleritis anterior nebst Iritis. Nach einem Rezidiv des Erysipels mit Beteiligung der anderen Gesichtsseite kam es auch auf diesem Auge zu Skleritis. Der infektiöse Weg dürfte

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COMPARISON OF THE ACTION OF 1-ETHYL THEOBROMINE AND CAFFEINE IN ANIMALS AND MAN

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Although 1-ethyl theobromine has long been known in chemical literature, study of its pharmacologic action has been meager indeed. The compound was first prepared by van der Slooten (1) in 1897, but nothing of its pharmacology was recorded until 1905, by Bergell and Richter (2). The latter authors concluded that 1-ethyl theobromine possessed a diuretic action about equal to caffeine, no mention of other actions being included in their report. Subsequently, only different methods for preparation of this substance were published.

The various xanthines are known to have similar effects on different functions of the body, but these actions vary in degree. Thus, theophylline, although superior as a diuretic, is less stimulating to respiration than caffeine. Stimulation of the central nervous system by caffeine has long been known and exhaustively studied in all aspects. In this respect, caffeine may be superior to benzedrine (3), but the diuretic action of the former is considered by some to be a slight disadvantage. Substitution of the methyl group in the 1-position of the caffeine molecule with other alkyl groups might enhance the stimulating effect on the nervous system while leaving the diuretic action unchanged or even decreased. In the present report, the pharmacology of caffeine is compared to its closest substitution product, 1-ethyl theobromine. The latter compound was generously supplied by Dr. L. P. Kyrides of the Monsanto Chemical Company, St. Louis.

GENERAL PROPERTIES. 1-Ethyl theobromine is a white crystalline substance, soluble in water and alcohol, slightly soluble in ether, and readily soluble in chloroform or acid water. It has a melting point of 165–166°C. The structural formula of the compound is the same as that of caffeine except an ethyl group is substituted for the methyl radical in the 1-position.

ACTION ON THE CENTRAL NERVOUS SYSTEM. *Convulsive action in mice.* Following injection into the tail vein of starved albino mice, 1-ethyl theobromine produced convulsions similar to those of caffeine; that is, mainly tetanic in nature. These convulsions were about 1 minute in duration, and if death did not result, the animals recovered rapidly. A period of increased motor activity (restlessness) was noticeable for 1 or 2 hours thereafter. Table 1 shows the results of a determination of the median convulsive dose as calculated by the method of Bliss (4). Caffeine was tested at the same time for comparison. 1-Ethyl theobromine appeared to be about 50 per cent more stimulating to the central nervous system than caffeine, as judged from dosage.

Studies in human beings. Six healthy young adults acted as subjects to test the effect of 1-ethyl theobromine on finger-tapping rate and critical fusion fre-

quency of flicker. According to Simonson and Enser (5, 6), finger-tapping rate and critical fusion frequency measure the function of motor centers and visual centers, respectively. These tests also have been employed by Steinhaus and Kelso (7) in studies of efficiency of human beings. Furthermore, Simonson and associates (8, 9) have shown that amphetamine and pervitin produce increased scores in these tests, while alcohol (10) has a depressant effect. Thus, it appears that these procedures measure the degree of efficiency of the central nervous system.

The 6 subjects were trained for more than a month before the actual test period. The tests were run on 9 consecutive working days (Monday to Friday, inclusive). The subject was told he would receive either 1-ethyl theobromine, caffeine, or a placebo, but did not know specifically which compound was to be given. The drugs were administered orally in gelatin capsules to avoid discovery

TABLE 1

The median convulsive doses of 1-ethyl theobromine and caffeine by intravenous injection into mice

DRUG	DOSE	NUMBER CONVULSED/ NUMBER USED	CD_{50} ± S.E.
	mg. per kg.		*mg. per kg.*
1-Ethyl Theobromine	50	3/10	54.1 ±2.0
	56	6/10	
	62	8/10	
	80	10/10	
Caffeine	50	0/5	84.4 ±4.9
	80	4/10	
	100	8/10	
	125	10/10	

by taste. The dosage used was 200 mg. in each case. After the subject took the capsule at 8:00 a.m., hourly tests were run throughout the day until 4:00 p.m., inclusive. Subjects were not allowed to have coffee, tea, or other caffeine-containing beverages on test days. All individuals continued their usual work in the laboratories during the experimental period.

The results of the finger-tapping tests are shown in table 2. Both 1-ethyl theobromine and caffeine produced consistently greater scores. The differences between treatments (placebo, 1-ethyl theobromine, and caffeine) were highly significant when submitted to an analysis of variance (11). Furthermore, caffeine was somewhat more effective than 1-ethyl theobromine in stimulating the rate of finger-tapping.

In table 3 are seen the effects of these compounds on critical fusion frequency of flicker. Here the mean values obtained with both 1-ethyl theobromine and caffeine were always greater than those of the controls. Statistical analysis also showed these differences to be highly significant. Caffeine was found again to be more stimulating than 1-ethyl theobromine in equal dosage.

more, a tracheal cannula was inserted and connected to a double valve. The dog was allowed to inspire only fresh air, while all expired air flowed through a modified Pavlov automatic, electro-magnetic clamp (12) into 1 of 2 small spirometers. The apparatus was so constructed that when one spirometer became filled, the Pavlov clamp immediately directed the flow of expired air into the other spirometer. While 1 spirometer was filling, the other spirometer was being evacuated. Each filling of a spirometer was automatically recorded by a signal magnet. This arrangement allowed a quantitative and continuous analysis of respiratory volume, which was necessary in comparing the action of 2 closely related compounds. The tambour method of recording respiration is only semi-quantitative.

Once the dog and apparatus were connected, repeated small doses of sodium phenobarbital were given until respiration was greatly depressed. The experi-

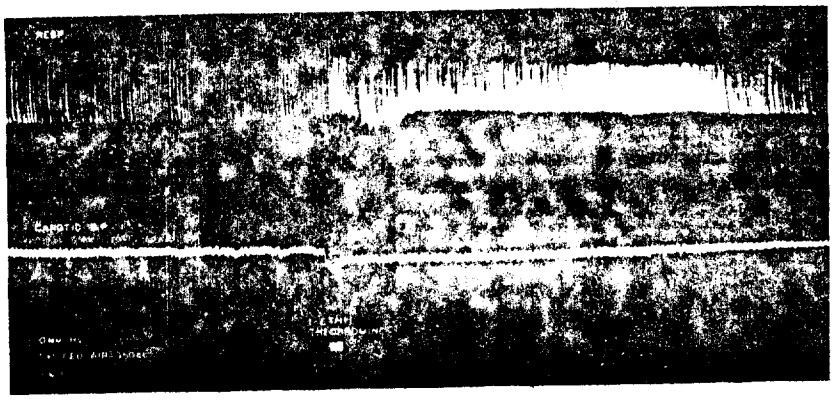

FIG. 1. RESPIRATORY STIMULATION PRODUCED BY 1-ETHYL THEOBROMINE

A female dog, weight 5.0 kg., was anesthetized with 150 mg. per kg. of sodium phenobarbital intravenously. An additional 40 mg. per kg. of the barbiturate was given, resulting in considerable respiratory depression. The effect of administering 1-ethyl theobromine, 10 mg. per kg. intravenously, is shown. Note the quantitative increase in volume of expired air.

ment was then allowed to run for 60–90 minutes without manipulation. During this control period, respiration was highly regular and recovery from anesthesia was not apparent. Intravenous injection of either 1-ethyl theobromine or caffeine was made at the end of the control period. A dose of 10 mg. per kg. was used in all tests. By this procedure, a total of 10 dogs was studied. Only 1 drug was given to each animal in order to avoid any conflict of action. A record of the effect of 1-ethyl theobromine is shown in figure 1. In table 4 it is obvious that caffeine produced less respiratory stimulation than 1-ethyl theobromine.

DIURETIC ACTION. *Effects in unanesthetized dogs.* Six female dogs were trained to lie quietly on a table following insertion of an in-lying catheter. On test days 1 hour previous to catheterization, 500 cc. of 0.9 per cent sodium chloride solution was administered by stomach tube since the urine output was otherwise too irregular and too small. This dose was equivalent to approximately 50 cc. per kg. Lipschitz and associates (13) used 25 cc. per kg. of isotonic sodium chloride solution in their assays of diuretics in rats. Isotonic saline is known to have little or

no diuretic action of its own when given orally (14). Animals were starved 18 hours prior to the actual test period. Following catheterization, the urinary bladder was emptied at 15-minute intervals. After 45–60 minutes when the urine output was quite regular, an intravenous injection of 10 mg. per kg. of either 1-ethyl theobromine or caffeine was made. The bladder was emptied every

TABLE 4

Comparison of the respiratory stimulating actions of 1-ethyl theobromine and caffeine by intravenous injection into dogs

DRUG AND DOSE	RESPIRATORY VOLUME DURING 30-MINUTE CONTROL PERIOD	RESPIRATORY VOLUME DURING 30 MINUTES FOLLOWING DRUG	PER CENT INCREASE OVER CONTROL	AVERAGE PER CENT INCREASE OVER CONTROL
10 mg. per kg.				
1-Ethyl Theobromine	2.56*	4.95*	93.4	38.4
	4.13	5.32	28.8	
	3.58	4.40	22.9	
	4.40	4.95	12.5	
	2.93	3.94	34.4	
Caffeine	3.30	5.41	63.9	15.8
	2.93	2.48	−15.4	
	3.94	4.40	11.7	
	3.76	3.76	0.0	
	2.93	3.48	18.8	

* All figures in these columns represent volume of expired air in liters per 5 minutes.

TABLE 5

The diuretic action of 1-ethyl theobromine and caffeine by intravenous injection into trained unanesthetized dogs

DOG	TOTAL OUTPUT OF URINE FOR 90 MINUTES FOLLOWING ADMINISTRATION OF DRUG		
	None	1-Ethyl Theobromine*	Caffeine*
	cc.	*cc.*	*cc.*
1	85.3	86.0	108.0
2	41.0	143.0	114.0
3	82.5	111.5	82.0
4	52.0	171.0	47.5
5	88.0	100.0	84.0
6	26.5	72.5	57.5
Mean	62.5	114.0	97.2

* Dose was 10 mg. per kg. in each case.

15 minutes for 1½ hours thereafter. In table 5, it is seen that 1-ethyl theobromine produced greater diuresis than caffeine. The value for the former was statistically significant in comparison with untreated animals. In the case of caffeine, the increased urine output was not quite significant by statistical analysis. It appeared that in dogs 1-ethyl theobromine was a stronger diuretic than caffeine.

Diuretic action in human beings. 1-Ethyl theobromine and caffeine were

tested for diuretic action in 6 healthy young adults. The following procedure was used, which is similar to that used by Keith (15) except that weighed diets were not used. Subjects were instructed to eat their regular diets and to avoid unusual foods. Furthermore, all salty foods were prohibited and a minimum amount of salt was to be added as seasoning. Water intake was limited to 1500 cc. per day, no caffeine-containing beverages being permitted. These individuals were all laboratory workers and continued their usual duties during the test days. The urinary bladder was emptied at 8:00 a.m. Immediately afterwards a capsule containing 200 mg. of one or the other drug or a placebo was taken orally. Thereafter, until 4:00 p.m. all urine specimens were collected and measured. The bladder was again emptied at 4:00 p.m. and the total urine output recorded for the day's test period. This procedure was followed for 9 consecutive working days. Each drug and placebo were tested on 3 separate days. The results of

TABLE 6

The influence of 1-ethyl theobromine and caffeine on urine output in human beings. Oral administration

SUBJECT	TOTAL URINE OUTPUT FOR THREE TEST PERIODS		
	Placebo	1-Ethyl Theobromine*	Caffeine*
	cc.	*cc.*	*cc.*
O. M.	1678	1615	1717
A. O.	1415	1605	1430
O. R.	1780	3045	2245
W. W.	730	2035	1315
N. M.	1255	2190	1470
M. E.	896	1373	1152
Mean daily output	431	659	518

* Dosage was 200 mg.

this diuretic study are shown in table 6. 1-Ethyl theobromine produced somewhat greater diuresis than caffeine. Statistical analysis revealed a highly significant difference between the 3 treatments.

TOXICITY STUDIES. *Acute toxicity in rats and mice.* The median lethal dose of 1-ethyl theobromine was determined in starved albino rats and starved albino mice. Both intravenous and oral routes of administration were used in rats, while mice received the drug by vein only. Calculations were made by the method of Bliss (4). The acute toxicity of caffeine was obtained simultaneously for comparison. Tabulation of results is found in table 7. Caffeine was about 30–50 per cent less toxic than 1-ethyl theobromine by this procedure.

Feeding experiments on rats. Employing the method of Anderson, Henderson, and Chen (16), young rats weighing about 100 gm. were fed a standard diet to which 1-ethyl theobromine was added. Six groups of 5 animals each were used, each group receiving a different percentage of 1-ethyl theobromine in the food. The percentages ranged from 0.02–0.5. A record was kept of the gain in weight

The acute median lethal doses of 1-ethyl theobromine and caffeine in albino rats and albino mice

ANIMAL	DRUG	ROUTE OF ADMINISTRATION	DOSE	NUMBER DIED/NUMBER USED	$LD_{50} \pm S.E.$
			mg. per kg.		*mg. per kg.*
Rat	1-Ethyl Theobromine	Oral	125	0/10	176 ±10.5
			160	5/10	
			200	8/10	
			250	12/15	
			300	10/10	
	Caffeine	Oral	200	2/9	233 ±14.0
			250	7/10	
			300	8/10	
			365	10/10	
	1-Ethyl Theobromine	Intravenous	56	0/8	73.6 ± 3.67
			62.5	4/10	
			70	5/10	
			80	6/10	
			90	7/10	
			110	3/3	
	Caffeine	Intravenous	100	2/10	104.8 ± 1.87
			110	8/10	
			125	9/9	
Mouse	1-Ethyl Theobromine	Intravenous	50	2/10	61.04 ± 2.41
			56	3/10	
			62	4/10	
			80	10/10	
	Caffeine	Intravenous	50	0/5	100.9 ± 6.67
			80	2/10	
			100	5/10	
			125	8/10	

TABLE 8

The effect on growth of feeding 1-ethyl theobromine and caffeine to young rats for 28 days

DRUG	CONCENTRATION OF DRUG IN FOOD	TOTAL WEIGHT GAIN OF 5 RATS
	per cent	*gm.*
1-Ethyl Theobromine	0.020	477
	0.035	343
	0.070	408
	0.100	348
	0.300	216
	0.500	136*
Caffeine	0.020	511
	0.035	406
	0.070	296
	0.100	436
	0.300	282
	0.500	54

* One rat died on the fifteenth day of the test.

of each animal. This regime was maintained for 28 days, at the end of which time all animals were sacrificed. An identical procedure was carried out with caffeine for comparison. In table 8 are shown the results. Statistically, there was no difference in the 2 treatments. There was little or no depression of growth with concentrations of either compound up to and including 0.1 per cent. Larger amounts of either drug inhibited weight gain somewhat. One rat died the fifteenth day of the test period, this death occurring in the group fed the greatest percentage of 1-ethyl theobromine.

Post-mortem examination was made of all rats which received the 0.5 per cent concentration of 1-ethyl theobromine or caffeine. No gross or microscopic damage was found in the animals which were given 1-ethyl theobromine, with one exception. The animal which died after 15 days on the diet showed pathological changes associated with emaciation. Of the 5 rats on caffeine, 3 were normal, 1 revealed slight pulmonary edema and congestion, and in the other animal hydronephrosis of the right kidney was noted but no stones were seen.

CONCLUSIONS

1. 1-Ethyl theobromine in sufficient doses produced stimulation of the central nervous system as evidenced by: (a) convulsions in mice, (b) increase in both critical fusion frequency of flicker and finger-tapping rate in adult human beings, and (c) augmentation of respiration in dogs deeply anesthetized with sodium phenobarbital.

2. 1-Ethyl theobromine appeared to be less potent than caffeine on the nervous system of human beings, but the former was more convulsant in mice and was a stronger respiratory stimulant in dogs.

3. No untoward effects were noted from an oral dose of 200 mg. of 1-ethyl theobromine taken by 6 adults each for 3 separate days.

4. 1-Ethyl theobromine had a diuretic action in human beings and dogs, this action being stronger than that of caffeine.

5. In mice and rats, the acute toxicity of caffeine, orally or intravenously, was 30–50 per cent less than that of 1-ethyl theobromine.

6. Prolonged administration of 1-ethyl theobromine in growing rats' food did not inhibit growth even when concentrations as high as 0.1 per cent were used. Greater amounts depressed growth, but little or no pathological changes occurred with quantities as great as 0.5 per cent of the food. No difference from caffeine was noted.

Acknowledgment. All statistical analyses were made by Mr. John C. Hanson. The feeding experiments in rats were carried out by Mr. Robert C. Anderson, and the pathologic examinations, by Dr. Paul N. Harris. To all three, we are greatly indebted.

BIBLIOGRAPHY

1. VAN DER SLOOTEN, W., Ueber einige Abkömmlinge des Theobromins. Arch. der Pharm., 235: 469, 1897.
2. BERGELL, P., AND RICHTER, P. F., Experimentelle Untersuchungen über die Beziehung zwischen chemischer Konstitution und diuretischer Wirkung in der Puringruppe. Ztschr. f. exper. Path. u. Therap., 1: 655, 1905.

3. Foltz, E. E., Ivy, A. C., and Barborka, C. J., The influence of amphetamine (benzedrine) sulfate, *d*-desoxyephedrine hydrochloride (pervitin) and caffeine upon work output and recovery when rapidly exhausting work is done by trained subjects. J. Lab. & Clin. Med., 28: 603, 1943.
4. Bliss, C. I., The determination of the dosage-mortality curve from small numbers. Quart. J. Pharm. & Pharmacol., 11: 192, 1938.
5. Simonson, E., and Enzer, N., State of motor centers in circulatory insufficiency. Arch. Int. Med., 68: 498, 1941.
6. Simonson, E., and Enzer, N., Measurement of fusion frequency of flicker as a test for fatigue of the central nervous system. Observations on laboratory technicians and office workers. J. Indus. Hyg. & Toxicol., 23: 83, 1941.
7. Steinhaus, A. H., and Kelso, A., Improvement of visual and other functions by cold hip baths. War Med., 4: 610, 1943.
8. Simonson, E., Enzer, N., and Blankstein, S. S., Effect of amphetamine (benzedrine) on fatigue of the central nervous system. War Med., 1: 690, 1941.
9. Simonson, E., and Enzer, N., Effect of pervitin (desoxyephedrine) on fatigue of the central nervous system. J. Indus. Hyg. & Toxicol., 24: 205, 1942.
10. Simonson, E., Personal communication.
11. Snedecor, G. W., Statistical Methods. Third Edition, The Iowa State College Press, Ames, Iowa, 1940.
12. Barcroft, H., A mechanical stromuhr. J. Physiol., 67: 402, 1929.
13. Lipschitz, W. L., Hadidian, Z., and Kerpcsar, A., Bioassay of diuretics. This Journal, 79: 97, 1943.
14. Wright, S., Applied Physiology. Fifth Edition, Oxford University Press, New York, 1934.
15. Keith, N. M., The action of diuretics in the normal individual. This Journal, Proc. 42: 260, 1931.
16. Anderson, R. C., Henderson, F. G., and Chen, K. K., The rat as a suitable animal for the study of prolonged medication. J. Am. Pharm. A., 32: 204, 1943.

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Synthetic Caffeine

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The identity of a synthetic compound with a natural product is usually made by the determination of physical constants such as the melting point, mixed melting point, and elementary analysis. However, with the many pharmacologically active substances, such as hormones and vitamins, a biological assay has been employed to supplement chemical identification. This appears important and necessary, because sometimes the physical constants do not completely establish the absolute purity of the product. Recently, for example, there was encountered in this laboratory a sample of synthetic pyridoxine which answered the specifications of *New and nonofficial remedies* (*4*), but which proved to be much more toxic than the standard.

Synthesis of caffeine and theobromine was initiated by Fischer and his co-workers (*1*), and newer methods of synthesis, partial or complete, have been achieved by other investigators (*5*). Previously, we studied the synthetic alkyl derivatives of theobromine (*3*). As an extension of the above work, it occurred to us that pharmacological proof of identity of totally synthetic caffeine and commercial caffeine made from a natural intermediate was desirable. The latter is usually prepared by methylation of theobromine. Samples of both were generously supplied by L. P. Kyrides, research director of the Monsanto Chemical Company, St. Louis, Missouri. The melting point of commercial caffeine ranged between 234.5 and 237° C.; that of synthetic caffeine, between 234.5 and 237.5° C.; and the mixed melting point, between 235 and 237° C.

Two types of experiments were carried out—one, the measurement of the stimulation of the central nervous system in rats; the other, the comparison of toxicity in mice by intravenous injection. The central stimulating action was evaluated in spring-suspended cages according to the method of Schulte and co-workers (*2*). Ten rats, weighing between 230 and 280 grams, were employed. A fixed dose of 20 mg./kg. of each drug in 0.5 per cent solution was injected subcutaneously. The observation period was exactly 4 hours, counting from the time of injection. Five animals received synthetic caffeine, and the other 5, the commercial. On the following day a cross-over test was made—those receiving the synthetic caffeine the previous day received the commercial, and vice versa.

For the toxicity study, mice weighing from 14.6 to 19.3 grams were used. The concentration of the solution was the same as for rats (0.5 per cent). Both samples were run on the same day.

The results are unequivocal. The average number of revolutions recorded from the cages by the 10 rats for commercial caffeine was 34.8; for synthetic caffeine, 34.1; and that of the control test (without medication), 5.1. The median lethal dose (LD_{50}) by intravenous injection of commercial caffeine in 30 mice was found to be 79.36 ± 6.94 mg./kg. The LD_{50} of synthetic caffeine in 30 other mice was determined to be 75.51 ± 5.39 mg./kg. There is no significant difference between the two figures statistically.

Pharmacological results indicate, therefore, that commercial caffeine and synthetic caffeine have the same degree of central stimulation and toxicity.

References

1. FISCHER, E. *Ber. Dtsch. Chem. Gesellsch.*, 1882, 15, 453; 1897, 30, 2400; 1899, 32, 435; FISCHER, E., and BROMBERG, O. *Ibid.*, 1897, 30, 219; FISCHER, E., and ACH, F. *Ibid.*, 1898, 31, 1980.
2. SCHULTE, J. W., REIF, E. C., BACHER, J. A., JR., LAWRENCE, W. S., and TAINTER, M. L. *J. Pharm. exp. Therap.*, 1941, 71, 62.
3. SCOTT, C. C., and CHEN, K. K. *J. Pharm. exp. Therap.*, 1944, 82, 89; SCOTT, C. C., ANDERSON, R. C., and CHEN, K. K. *Ibid.*, 1946, 86, 113.
4. SMITH, A. *New and nonofficial remedies*. Chicago: American Medical Association, 1945. P. 620.
5. TRAUBE, W. *Ber. Dtsch. Chem. Gesellsch.*, 1900, 33, 3035; SCHMIDT, E., and PRESSLER, H. *Liebig's Ann. Chem.*, 1883, 217, 287; ENGELMANN, M. *Ber. Dtsch. Chem. Gesellsch.*, 1909, 42, 177; FIORE, G. *Boll. chim. farm.*, 1915, 54, 609; BEITZ, H., and DAMM, P. *Liebig's Ann. Chem.*, 1917, 413, 186; ROSENMUND, K. W., and ZETZSCHE, F. *Ber. Dtsch. Chem. Gesellsch.*, 1918, 51, 578.

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TOXICOLOGY AND APPLIED PHARMACOLOGY 10, 199–201 (1967)

COMMUNICATION

Effect of Caffeine on the Lethal Dose of Digoxin in Dogs[1]

Caffeine was reported to increase[2] or to reduce[3] the toxicity of digitalis in cats. The study by Kohn and Costopanagiotis provided evidence for the claim in one of the textbooks of pharmacology[4] that caffeine reduces digitalis toxicity.

Digoxin as well as caffeine has a positive inotropic action on the myocardium. This effect of both drugs may be associated with an increase in the availability of Ca^{++} for the contractile process.[6,5] We recently became interested in the mechanism by which caffeine modifies the toxicity of digitalis. The effect of caffeine on the lethal dose of digoxin was studied in dogs.

The experimental procedure was similar to that previously used in this laboratory.[7,8] Mongrel dogs, 6–14 kg body weight, were anesthetized with sodium pentobarbital, 25 mg/kg intravenously. Artificial respiration was maintained with a Harvard Respiratory Pump (25 cycles per minute, stroke volume 300–400 cm^3). The left femoral vein and artery were cannulated, arterial blood pressure was recorded on a Sanborn 150 Recorder through a Model 267A Sanborn Pressure Transducer. Digoxin[9] was dissolved in 70% ethyl alcohol to contain 0.50 mg/ml and further dissolved is saline in accordance with the body weight of the animal, so that 90 ml of infusion fluid contained the theoretical lethal dose of digoxin -0.25 mg/kg.

In the first series of experiments, caffeine sodium benzoate,[10] 50 mg/kg, was

[1] This work was aided by Institutional Grants, Philadelphia General Hospital, and the Foundation for Cardiovascular Research, Philadelphia, Pennsylvania.

[2] WIETHAUP, H. (1932). Die Beeinflussung der Digitaliswirkung durch koronarerweiternde Mittel. *Arch. Exptl. Pathol. Pharmakol.* **168**, 554–560.

[3] KOHN, R. and COSTOPANAGIOTIS, B. C. (1933). Zur experimentellen Veränderung der Digitalisgiftigkeit. II. Mitteilung: Die Digitaliskombination mit einigen Purinderivaten. *Arch. Exptl. Pathol. Pharmakol.* 170, 226–238.

[4] SOLLMAN, T. (1948). *A Manual of Pharmacology*, 7th ed., p. 460. Saunders, Philadelphia.

[5] KLAUS, W., and LÜLLMANN, H. (1964). Calcium als intracelluläre Überträgersubstanz und die mögliche Bedeutung dieses Mechanismus für pharmakologische Wirkungen. *Klin. Wochschr.* **42**, 253–259.

[6] NAYLER, W. G., and HASKER, J. R. (1966). Effect of caffeine on calcium in subcellular fractions of cardiac muscle. *Am. J. Physiol.* **211**, 950–954.

[7] BEYDA, E. J., JUNG, M., and BELLET, S. (1961). Effect of hypothermia on the tolerance of dogs to digitalis. *Circulation Res.* **9**, 129–135.

[8] TAKAGI, M., ZANUTTINI, D., KHALIL, E., and BELLET, S. (1965). Tolerance of reserpinized dogs to digitalis. *Am. J. Cardiol.* **15**, 203–205.

[9] Supplied as Lanoxin® powder through the courtesy of Dr. R. Maxwell, Burroughs-Wellcome & Co., Tuckahoe, New York.

[10] Caffeine sodium benzoate, EFCO LABORATORIES, Hickville, New York, in ampules, 0.25 g/ml.

given intramuscularly prior to anesthesia 1 hour prior to the start of digoxin infusion. In the experiments involving simultaneous infusion of both drugs, caffeine was added to the infusion fluid containing digoxin so that 90 ml of the medium contained 100 mg/kg of caffeine sodium benzoate; the total amount of infused caffeine sodium benzoate varied from 72 to 110 mg/kg.

The infusion rate was maintained in all experiments at 1 ml/min with either a Brewer pipetting machine or buret. During the first 30 minutes of the experiment, the blood pressure was recorded at 5-minute intervals and continuously thereafter. Electrocardiograms (lead II) were obtained at 5-minute intervals during the first 30 minutes of the experiment and at 2–5-minute intervals thereafter. The end point of all experiments was an abrupt fall in blood pressure accompanied by electrocardiographic evidence of ventricular fibrillation.

Caffeine sodium benzoate, 50 mg/kg intramuscularly, had no effect on the lethal dose of digoxin. The mean lethal dose of digoxin was 0.26 mg/kg with a standard error of ± 0.02 in seven control experiments and 0.27 mg/kg with a standard error of ± 0.01 in eight dogs pretreated with caffeine sodium benzoate.

In seven experiments involving simultaneous infusion of digoxin and caffeine sodium benzoate, the mean lethal dose of digoxin was 0.23 ± 0.01 mg/kg. The difference from the control was not significant at 95% level ($p = 0.1$).

In one additional exploratory experiment, caffeine sodium benzoate was administered at 25 mg/kg, and in another experiment at 100 mg/kg, intravenously, 5 minutes prior to the start of digoxin infusion. In both experiments, the lethal dose of digoxin remained within the range of that obtained in control experiments.

Analysis of the electrocardiograms revealed the usual toxic effects of digitalis in all experiments: prolongation of P-R interval, depression of S-T segment, as well as all forms of supraventricular, nodal, and ventricular premature beats, and paroxysmal ventricular tachycardia.

Knowledge of the nature of the interaction between digitalis and caffeine is not only of clinical, but also of considerable theoretical, interest. Both drugs have positive inotropic action, which may be mediated by a common mechanism. According to one of the newer theories, an increase in intracellular free Ca^{++} concentration is primarily responsible for the positive inotropic effect of digitalis. This increase in Ca^{++} is thought to be the result of either an increase in Ca^{++} release from endoplasmic storage sites or of a reduction in the binding of Ca^{++}.[5] At high doses, digitalis may interfere with the Ca^{++} efflux; this interference would also contribute to further increase in free intracellular Ca^{++}. The proarrhythmic activity of digitalis at toxic dose levels is, however, not likely to involve an increase in free intracellular Ca^{++}. This is probably determined by the digitalis-induced blockade of active Na^{+} and K^{+} transport through the myocardial cell membrane.[11]

Caffeine was observed to increase Ca^{++} influx as well as efflux in the frog

[11] WEATHERALL, M. (1966). Ions and the action of digitalis. *Brit. Heart J.* 28, 497–504.

sartorius muscle[12] and in toad ventricular muscle.[13] This is thought to be associated with a reduction in the binding of Ca^{++} in the membrane and myoplasm. According to Nayler and Hasker,[6] caffeine interferes with the uptake and with the release of Ca^{++} from isolated cardiac mitochondria. It is conceivable that more Ca^{++} may become available for the processes associated with excitation-contraction coupling and that an increase in the intracellular concentrations of free Ca^{++} may be responsible for the positive inotropic action of caffeine.

On the basis of these considerations, there is no reason to expect caffeine to antagonize the toxic effects of digitalis, and our results did not support such antagonism. Caffeine did not increase the lethal dose of digoxin in dogs. A slight reduction in the lethal dose of digoxin by caffeine, 100 mg/kg intravenously, was in our study not significant statistically. The possibility that caffeine at doses higher than employed in our experiments will significantly reduce the lethal dose of digoxin was not excluded. Such doses would, however, approach the lethal dose of caffeine, which was reported to be 175 mg/kg intravenously.[14]

Summary

Caffeine did not significantly alter the lethal dose of digoxin in dogs. The mean lethal dose of digoxin by intravenous administration to seven dogs was 0.26 mg/kg with a standard error of ±0.02; in eight animals pretreated intramuscularly with caffeine sodium benzoate, 50 mg/kg, the lethal dose of digoxin was 0.27 ± 0.01 mg/kg; and in seven dogs infused with digoxin and caffeine sodium benzoate mixture, it was 0.23 ± 0.01 mg/kg. These results did not substantiate the reported antagonism of digitalis toxicity by caffeine.

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[12] Bianchi, C. P. (1961). The effect of caffeine on radiocalcium movement in frog sartorius. *J. Gen. Physiol.* 44, 845–848.

[13] Nayler, W. G. (1963). Effect of caffeine on cardiac contractile activity and radiocalcium movement. *Am. J. Physiol.* 204, 969–974.

[14] Sollman, T., and Pilcher, J. D. (1911). The actions of caffeine on the mammalian circulation. *J. Pharmacol. Exptl. Therap.* 3, 19–92.

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IMMEDIATE EFFECTS OF CHEMICALS ON MOUSE CHROMOSOMES
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Mouse testes were cultured in a saturated solution of caffeine. The effects are positive: stickiness of the chromosomes, clumping of the chromosomes and chromatin extrusion from the nuclei – or negative: gap formation and fragmentation of the chromosomes and vacuolar extrusion of material from the nuclei.

In positive action caffeine acts on the extragenic DNA. In the negative action it destroys the Feulgen reactivity of the DNA, thus breaking down certain nucleotides. By influencing the supply of certain nucleotides caffeine may be involved in mutagenesis and chromosome breaks and their reunion.

1. Introduction

In the field of caffeine mutagenesis there is some confusion due to several contradictory reports. Most of them contain genetical and biochemical results and since 1949 (Kihlman and Levan, 1949) none of the recent ones is based on direct cytological and chromosomal observations.

The present note is concerned with morphologically definable and repeatable chromosomal effects of high dose of caffeine in the mouse testis. High dose was used, because it was thought that small doses may not produce clear cut results, testes were used, because they represent on organ in which nuclei of cells of somatic tissues as well as those of spermatogonial lineages can be simultaneously treated and observed in identical conditions.

The question asked is: what does the caffeine treatment do to the chromosomes of the mouse?

2. Material and methods

Adult mice were killed by etherisation, their testes were immediately removed from the body, immersed without disrupting, in the physiological salt solution (0.9% NaCl) containing caffeine and kept as a short term organ cultures at +35°C. Control material was kept in the salt solution without any addition. At the end of the experiment the material was fixed and squashed in acetocarmine and stained by the modified Feulgen technique. In the text below F+ and F– stand for Feulgen positive and negative material respectively.

Solubility of caffeine (Handbook of Chem. and Physics, 1962) in water depends on the temperature: no more than 1.35 g of caffeine is dissolved in 100 ml of water at +16°C, while at the temperature +35°C a saturated solution of caffeine contains about 20 g of the drug per 100 ml of water. Testes were kept in saturated (at +35°C) solution of caffeine in the amount of 1 ml per testis.

Caffeine is rapidly penetrating to all parts of the body; in 30 min after consumption it is in uniform concentration in the whole body (Goldstein and Warren, 1962).

Caffeine has metabolic half life of 3.5 hr (Axelrod and Reichenthal, 1953), which means that one single dose will be in 99% metabolised during 24 hr.

In the experiments reported here the testes were kept in caffeine solution for 25 min, 1 hr, 2, 4, 6, 16, 24, and 36 hr, control series were run parallelly for 6, 16, and 36 hr.

3. Results

No changes were detected in control material, except for some necrotic phenomena detectable first after 16 hr.

Generally the changes induced by caffeine are not equally detectable in all parts of the testis, nearer the surface or perhaps also nearer the blood vessels the changes are more expressed than elsewhere. Thus a slide may contain cells with extreme changes and almost normal cells as well. Changes as a rule increase in expression with the time of treatment but there is no uniform gradient of dose dependence detectable.

The effects of caffeine were found in the nuclei of the cells of membrana basalis of the tubuli seminiferi, of spermatogonia, of spermatocytes in successive stages and in the spermheads of the differentiating spermatozoa.

No changes were seen in the nuclei of the blood vessels nor in the nuclei of the Sertoli cells, except perhaps for smaller nucleoli in the latter ones (which however, was not statistically significant).

Nuclei of the cells of membrana basalis show the most unusual effect of caffeine (fig. 1). Normally these nuclei are elongated, oval and flat and contain about 18 equally spaced small chromocentres. After 6 hr of treatment small vacuoles appear near the nuclear surface. Later they apparently break open emptying their contents into the cytoplasm. The nucleus, however, does not return to its smooth outlines, but shows a permanent dent caused by the detachment of the vacuole. The contents of the vacuole are F–, while the remaining part of the nucleus retains its slightly F+ nature. By 16 hr or more the larger vacuoles are formed and the dents left by them are large and crescent shaped. The chromocentres appear normal and do not alter their position inside the nucleus, thus they disappear if they happen to be engulfed by the vacuole. Eventually the walls of the dents from all sides come near to each other and the nucleus, now containing a reduced number of chromocentres assumes a shape of an irregular polyhedron with concave sides. Finally the nuclei are no longer recognisable forming an undefined debris.

In the interphase nuclei of the spermatogonial cells after 25 min of treatment come clumping of the chromosomal material is seen and after 2 hr there is a general clumping affecting most of the nuclei. In many of these an extrusion of F+ material in the form of "globules on stalk" sticking out from the clumped mass can be seen (fig. 2). Due to the clumping of the chromosomes mitotic figures are not easily recognisable. Some nuclei, from 4 hr of treatment onwards, show typical achromatic gaps in their chromosomes. These nuclei are in a high degree of "clumpiness", their chromosomes form a fused mass from which some chromosomal limbs, obviously less affected by clumping, are sticking out; it is in them that the gaps can be seen.

In the spermatocytes during the prophase similar gaps appear frequently (fig. 3). They do not interfere with zygotene pairing nor with later progress of the prophase. The gaps in some spermatocytes become longer at the same time the intergap segments stain more intensely until finally the pachytene chromosomes disintegrate into numerous strongly staining fragments of different sizes. Frequently among these fragments there is one very large with a shape of an elongated pear (fig. 4).

There is no clumping in the very infrequent diplotene and diakinesis stages, the bivalents sometimes show uneven stainability, however, the chiasmata behave normally in regard to their frequency and movements.

In the course of meiosis after 25 min of treatment there is the same type of chromosome clumping as in mitotic divisions, and by 6 hr there is the same difficulty in identifying of the metaphases of the first and of the second meiotic division.

Spermheads of the developing spermatozoa show another type of unusual change (fig. 5). They loose their stainable substance. There appears to be some pattern of the reduction of the F+ materials in them: first a non-stainable (F–) line or slit is visible in the spermhead, later it widens and other similar slits are formed. A transparent vacuole (F–) can sometimes be observed at the wider end of the spermhead. The whole process may lead to "ghost" spermheads with remnants of narrow lines of F+ material.

From the results, the initial question can be answered: caffeine does affect the normal behaviour of the F+ materials in the nuclei of certain cells, caffeine does not primarily affect the movement of the chromosomes.

Fig. 1. a. Nucleus of Sertoli cell; b. nuclei of spermatogonial cells; c. vacuolar extrusion of Feulgen negative material from the nuclei of cells of membrana basalis; d. nuclei of the cells of blood vessels. 1000 X.
Fig. 2. Chromatin extrusion from the nuclei of spermatogonial cells. 1000 X.
Fig. 3. Gaps in pachytene chromosones. 1000 X.
Fig. 4. Disintegration of pachytene nuclei, 1000 X. a. Traces of pachytene organisation; b. "elongated pear" fragment.
Fig. 5. Loss of Feulgen positive material from the spermhead of the tubular spermatozoa. 1000 X.

4. Discussion

Kihlman and Levan (1949) working with *Allium* were first to describe the chromosomal effects of caffeine. Immediately after the start of the treatment some chromosomes became sticky. Another reaction appearing also very soon was in the form of the "pseudochiasmata" which, as the authors put it: "indicate that certain points of anaphase chromosomes are unable to separate". In a series of plants treated for 24 hr and then cultured in pure water there was no stickiness but there were many structural changes like fragments, sister chromatid reunions, translocations etc., and at the highest dose (0.4%) also erosions which could lead to chromosomal lesions.

There can be little doubt that stickiness is a forerunner of clumpiness as are the gaps of fragmentation, and that both are the result of the regionally different amount of F+ material. Thus putting it in a general way – the main feature of the action of caffeine in the field of chromosomes is the "either or" change of the normal behaviour of the F+ material: there is either positive effect, when excessive F+ materials accumulate on the chromosomes giving stickiness, pseudochiasmata and clumping, – or there may be a negative action with a reduction of F+ materials producing gaps, fragmentation and disintegration. It is known that the positive effect is reversible (Kihlman and Levan, 1949), but nothing is known about the reversibility of the negative effect, except perhaps, the reversible arrest of cell divisions (which is compensated after the removal of caffeine) or the opposite: the irreversible damage to the nuclei of membrana basalis.

With the twofold action of caffeine a regional differentiation of the chromosomes can be visualized. It is possible that there may be some chromosome inherent cause for it like e.g. regional differences in AT/GC ratio. The "elongated pear" fragment indicates that in pachytene stage in different nuclei often the same region accumulates the F+ material in the same manner. This supports the idea of the inherent cause for deciding whether the effect will be positive or negative.

That genetic constitution may influence the action of caffeine can be suspected in the case reported by Clark and Clark (1968). When *Drosophila melanogaster* females of the constitution yw^a were treated with caffeine there were more males among their offspring, and when the females were of the *asc* constitution there were less males. The shift in the sex ratio was highly significant. The phenomenon could perhaps be explained by assuming that temporary "lethal" action of caffeine is more efficient when the locus *bb* is partitioned by inversion in the *asc* females, and less efficient when locus *bb* is in one block at the base of the X chromosome in the yw^a females.

The whole nuclei can be uniformly affected by negative (nuclei of membrana basalis) or by positive (total clumping) action of caffeine. In these cases the state of the synthetic activities prevailing at the time in the nucleus may responsible for the course of action of caffeine.

The positive action of caffeine depends on the existence of the extra chromosomal DNA. The existence of non-genic DNA does not militate with the fact that genes are made of the DNA. There may be a species-constant relation between the genic and non-genic DNA, which could explain big differences between closely related species (for example: Villiger et al., 1970). Sampson and Davies (quoted by Villiger et al., 1970) found a metabolically labile (non-genic) DNA besides the stable one. Walker (1968) described in the nuclear DNA of the mouse two distinct fractions – one of them (satellite DNA) contains 34% G/C as compared with average of 42% for the mouse. Variation in the size of the chromosomes in related species and among tissues of the same organism (in Darlington, 1937), between the nuclei of the same tissue (Slizynski, 1967), well known F+ structureless connections between non-homologous regions of polytene chromosomes and the reduction of the contents of DNA in the sperm by 35% on the 10-th day of storing (Salisbury, 1961) – these are facts which may be quoted as evidence for the existence of the extra-genic DNA. The extra-genic DNA may come from the nuclear sap, where according to Izawa et al. (1968), there is DNA in the amount comparable to that of the chromosomes or it may be a by-product or a surplus from an unscheduled synthesis.

The positive and negative action of caffeine can proceed simultaneously in the same nucleus as is the case with pachytene chromosome disintegrating. It is possible that caffeine may induce in the interphase

chromosomes abnormal sequences of activations that may be related to the teratogenicity of caffeine (Nishimura and Nakai, 1960). It would be of interest to see whether the chromocentres in differentiating cells can be influenced by caffeine.

The negative changes in which the F+ material disappears from the chromosomes may lead to chromosome breaks. Kihlman and Levan (1949) enumerated among the effects of caffeine in *Allium* many chromosomal structural changes due to the breakage and rejoining of the chromosomes.

Cattanach (1962) discusses the possibility for meiosis in the mouse – while Kuhlmann et al (1968) report the fact for mammalian cells in vitro – that chromosome breaks caused by caffeine have lost the ability to rejoin.

It is difficult to reconcile these two contradictory findings unless it is assumed that plant chromosomes differ from those of mammalians. A suggestive case (not necessarily directly applicable to the point in question) of differences between plant chromosomes and those of mammalians form the inversions connected with a change of polarity. They are very frequent in plants and in *Drosophila*, but, in spite of their good detectability in meiosis, are totally absent from mammalian caryology.

Under the influence of caffeine there is a great reduction of the volume of the nuclei of membrana basalis due to the extrusion of materials. This extrusion includes various substances and certainly the DNA of the engulfed chromocentres. The contents of the vacuoles are F–; similarly F+ material from the spermheads is removed in the form of unorganized F– substance. Both these observations show that caffeine in its negative activity causes changes in the sugar part of the DNA resulting in the loss of stainability and in the disintegration of the organized DNA. Caffeine appears to reveal two types of DNA – one which remains F+ and is extruded in the form of globules or fragments and the other which becomes F– and is extruded in the form of vacuoles.

Finally a suggestion can be put forward on the caffeine mutagenesis. A mutational change in a triplet, a substitution of new, different base pair for the old one, has to spread to all homologous triplets in the entire locus, otherwise the mutation will not be stable and complete. This spreading may depend on the number of conditions among which the most important is the availability of new bases. This may be influenced by caffeine, which could thus be regarded as indirectly involved in the mutagenesis and replication and in the rejoining of the broken chromosome ends.

5. Summary

Mouse testes were cultured in a saturated solution of caffeine. The effects are positive: stickiness of the chromosomes, clumping of the chromosomes and chromatin extrusion from the nuclei – or negative: gap formation and fragmentation of the chromosomes and vacuolar extrusion of material from the nuclei.

The choice of the effect may depend on the state of the nucleus and on the regional characteristics of the chromosomes.

In positive action caffeine acts on the extragenic DNA. In the negative action it destroys the Feulgen reactivity of the DNA, thus breaking down certain nucleotides. By influencing the supply of certain nucleotides caffeine may be involved in mutagenesis and chromosome breaks and their reunion.

References

Axelrod, J and J.Reichenthal, 1953, The fate of caffeine in man and a method for its estimation in biological material, J. Pharm. Exp. Therapy 107. 519.

Cattanach, B.M., 1962, Genetical effects of caffeine in mice, Z. Vererb. 95, 215.

Clark, P.M. and E.G.Clark, The genetic effect of caffeine in *Drosophila melanogaster*, Mut. Res. 6, 227.

Darlington, C.D., 1937, Recent advances in cytology (London) p. 55 and p. 78.

Goldstein, A. and R.Warren, 1962, Passage of caffeine into human gonadal and fetal tissues, Bioch. Pharmacol. 11, 166.

Handbook Chem. and Physics, 1962, 44-th edition (Hodgman, Cleveland, Ohio).

Izawa, M., V.G.Alfrey, and A.E.Mirsky, 1963, Composition of the nucleus and the chromosomes in the lampbrush stage of the newt oocyte, Proc. Nat. Acad. Sci. 50, 811.

Kihlman, B.A. and A.Levan, 1949, The cytological effects of caffeine, Hereditas 35, 109.

Kuhlmann, W., H.G.Fromme, E.M.Heege, and W.Ostertag, 1969, The mutagenic action of caffeine in higher organisms, Cancer Res. 28, 2375.

Nishimura, H. and K.Nakai, 1960, Congenital malformations in offspring of mice treated with caffeine, Proc. Soc. Exp. Biol. 104, 140.

Salisbury, M., 1961, Physiology of reproduction and artificial insemination of cattle (London) p. 493.

Slizynski, B.M., 1967, DNA and genes, Nature 214, 112.

Villiger, M., C.Czihak, P.Tardent, and F.Baltzer, 1970, Feulgen microspectrometry of spermatozoa and blastula nuclei of different sea urchin species, Exp. Cell Res. 60, 119.

Walker, P.M.B., 1968, How different are the DNA from related animals? Nature 219, 228.

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STUDIES ON THE TERATOGENIC INFLUENCE OF CAFFEINE ON WHITE MOUSE FETUSES

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Developmental disorders observed in white mouse fetuses after treating the pregnant females with caffeine in doses of 0.25—0.8 mg/kg body weight between the 7th and 14th days of pregnancy are described. Developmental disorders in the extremities predominated.

INTRODUCTION

In recent years, new drugs with well defined effects on the body have been introduced in therapy, many of which, however, have unforeseen harmful side effects. In some cases the symptoms of these effects are irreversible, e.g. teratogenic effects (2). This also applies to some specific drugs widely used in the past, such as caffeine (1, 3, 5). Very few papers devoted to the teratogenic action of caffeine have appeared in the literature. In 1929, *Stieve* noted that administration of large doses of caffeine to pregnant rabbits is the cause of death of embryos, respectively retarded fetal development. Eleven years later, these findings were confirmed by *Fischer* (cited after 3).

In 1938, *Druckrey* and *Szreiber* (cited after 3) observed cessation or irregularity of mitosis in the fertilized ova of sea urchins after they were immersed in a solution of caffeine. *Nishimura* and *Nakai* (3) injected caffeine in doses of 0.25 mg/kg body weight intraperitoneally in pregnant mice, resulting in death of the embryos or fetuses. *Bertrand* et al. (1) induced developmental anomalies in rats by injecting caffeine in doses of 75—150 mg/g body weight. Similar results were obtained in mice.

We have not been able to find any experimental studies on the teratogenic effects of caffeine in the available Polish literature.

The purpose of this study was to demonstrate the teratogenic effect of caffeine on white mouse fetuses. The choice of caffeine was dictated by the wide use of this compound in therapy, and especially in condiments such as coffee and tea.

MATERIAL AND METHODS

Experiments were carried out with 122 pregnant white mice, personally bred. The animals were fed with granulated fodder and carrots, and received drinking water ad lib. Throughout the duration of the experiment the mice were kept under conditions of constant temperature and illumination. The mice were fertilized naturally, the appearance of a vaginal plug being taken as the first day of pregnancy. Fertilized females were separated from males. The pregnant mice were divided into 8 groups. Sixteen mice of the control (first) group were given a single subcutaneous injection of 0.4 ml of 0.9% NaCl solution between the 7th—14th days of pregnancy. In groups 2—8, caffeine was injected between the 7th—14th days of pregnancy in doses of 0.25, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 mg/g body weight. The injections were always made at the same time of day. After injection of caffeine, natural delivery was awaited, which occurred on the 20th day of pregnancy, or autopsies were performed on the 19th day after fertilization. In cases in which resorption was anticipated, autopsies were performed earlier. Dead fetuses were transferred directly to 90° alcohol, and living neonates were left to develop further. Each fetus was first examined carefully with a stereoscopic microscope. Young mice in which gross developmental disorders were observed were killed after 2 weeks, and the skeletal system was stained with alizarin red according to *Spalteholz* (4). Stillborn and partially resorbed fetuses were also stained with alizarin red.

RESULTS

Group I (controls)

All the females of this group gave birth in term to neonates without any visible developmental disorders (Figs. 1, 7).

Group II

The animals of this group received a single injection of caffeine in doses of 0.25 mg/g body weight between the 7th and 14th days of pregnancy. In 8 out of 24 mice, resorption of fetuses was observed, especially when caffeine was injected on the 7th or 8th days of

Fig. 1.

Fig. 2.

Fig. 1. Control preparation of the skeletal system of a 14-day mice stained by the alizarin method.

Fig. 2. Skeletal system of a mouse fetus after injection of caffeine in the pregnant mouse in doses of 0.3 mg/g body weight on the 12th day of pregnancy. Numerous developmental anomalies in the skeletal system. Stained by the alizarin method.

pregnancy, and less often on the 9th, 10th, 11th and 12th days; in mice injected on the 13th and 14th days of pregnancy resorption of fetuses was not observed. The size of the resorbed fetuses varied. The remaining 16 mice of this experimental group gave birth to normal neonates in term without visible developmental disorders.

Group III

Out of 24 mice treated with caffeine in the dose of 0.3 mg/g body weight, resorption of fetuses occurred in 7, and 2 mice gave birth to live and dead fetuses with numerous developmental disorders (Figs. 2, 8); the remaining pregnant mice gave birth to neonates without visible developmental anomalies. Resorption was observed after injections of caffeine on the 7th and 8th days of pregnancy.

Group IV

This group of pregnant mice was treated with caffeine in doses of 0.4 mg/g body weight. In 6 mice which were given injections on the

Fig. 3.

Fig. 4.

Fig. 3. Skeletal system of a 13-day mouse after injection of caffeine in the pregnant mouse in doses of 0.4 mg/g body weight on the 11-th day of pregnancy, showing developmental anomalies of the left anterior extremity. Defect of the radius and shortening of the ulna. Lateral curvature of the palms at an angle of 90° with 3 normal digits.

Fig. 4. Left palm of 1-day after injection of caffeine in the pregnant mouse in doses of 0.4 mg/g body weight on the 10th day of pregnancy. Fusion and shortening of all the digits and lack of the metacarpal bones. Stained by the alizarin method. Magn. approx. × 30.

7th and 8th days of pregnancy intrauterine death of fetuses was observed. The progeny of the remaining 18 mice treated with caffeine between the 9th and 14th days of pregnancy exhibited various developmental disorders (Figs. 3, 4, 5, 6, 9, 11 and 12).

In a majority of cases, subcutaneous hematomas accompanied developmental disorders. The types of developmental disorders observed in this group are shown in Table I.

Group V

The animals of this group received caffeine in doses of 0.5 mg/g body weight. Resorption of fetuses was observed in 7 pregnant mice; in 4 autopsied cases on the 19th day of pregnancy live and dead fetuses with numerous developmental disorders were extracted. In the remaining cases, the pregnant females died several hours after injection of

Table I. Numbers and types of developmental disorders observed in the progeny of mice which were treated during pregnancy with caffeine in doses of 0.[illegible] mg/g body weight

Day of injection	No. of fetuses with developmental disorders	No. of fetuses with developmental disorders								
		Digital deformities*)		Shortened extremities	Disorders of muscular development	Cleft palate	Harelip	Astomia	Jaw defects	Others
		Anterior extremities	Posterior extremities							
9	24	10	24	—	—	—	—	—	—	—
10	13	13	4	—	—	—	—	—	—	—
11	21	12	—	6	—	—	—	—	—	3**
12	18	11	5	—	12	18	—	18	—	—
13	26	4	18	—	20	26	3	19	8	2***
14	25	24	—	—	—	—	—	—	—	—

*) brachydactyly, arachnodactyly, actrodactyly, syndactyly, hypophalangism

**) tail curvatures

***) curvatures of the spinal column

caffeine. Resorption of fetuses occurred irrespective of the day of injection — after 7 as well as 14 days of pregnancy. Developmental disorders were observed in the progeny of mice in which caffeine was injected exclusively on the 11, 12, 13 and 14th days of pregnancy. The disorders were similar to those observed in group IV. In many cases, subcutaneous hematomas were noted in the neighborhood of the developmental disorders.

Groups VI, VII and VIII

The animals of these experimental groups received injections of caffeine in doses of 0.6, 0.7 and 0.8 mg/g body weight. The mice which received injections of 0.6 and 0.7 mg/g died within several hours. In group VIII, all the mice died within several minutes after caffeine injections amid clonic-tonic convulsions.

DISCUSSION OF THE RESULTS

In a proportion of cases, administration of caffeine to pregnant mice caused intrauterine death of embryos and fetuses or developmental disorders in the progeny. Caffeine injected in doses of 0.35 mg/g body

weight caused intrauterine death and resorption of fetuses in 33% of the mice. Doses of 0.3 mg/g body weight caused resorption of embryos or fetuses in 30% of cases, and in 8% developmental anomalies. Doses of 0.4 mg/g body weight were attended by a further increase in the proportion of fetuses with developmental disorders. In this experimental group, developmental disorders were observed in 73%, and resorption of embryos and fetuses in 27% of cases.

Fig. 5. Fig. 6.

Fig. 5. Part of the anterior extremity of a 1-day mouse after injection of caffeine in the pregnant mouse in doses of 0.4 mg/g body weight on the 12th day of pregnancy. Palm with 4 fingers. Stained by the alizarin method. Magn. approx. × 30.

Fig. 6. Part of the posterior extremity of a 1-day mouse after injection of caffeine in the pregnant mouse in doses of 0.4 mg/g body weight on the 13th day of pregnancy. Disorders of ossification of the phalanges. Stained by the alizarin method. Magn. approx. × 30.

Administration of caffeine in the above-mentioned doses in the early phase of embryonic development, i.e. on the 7th and 8th days of pregnancy, usually caused intrauterine death of the embryos. The frequency of resorptions was smaller on the later days after fertilization. Owing to the rapid progress of resorption, the cause of the intrauterine death of embryos and fetuses could not be determined. This interesting phenomenon deserves further study and observations.

than 0.5 mg/g body weight caused death of 100% of the pregnant mice, regardless of the weight of the animals.

The developmental disorders predominated in the skeletal system of the extremities, especially in the digits. Disorders such as brachydactyly, arachnodactyly, syndactyly, ectrodactyly, hypophalangism and brachymely were observed. Cleft palate, maxillary defects and defects of the cranial vault were encountered less often. Besides skeletal defects, disorders in the development of the soft parts were noted, such as astomia and harelip.

Fig. 9.

Fig. 10.

Fig. 9. One-day mouse after injection of caffeine in the pregnant mouse in doses of 0.4 mg/g body weight on the 13th day of pregnancy. Defect of the left part of the maxilla.

Fig. 10. One-day mouse after injection of caffeine in the pregnant mouse in doses of 0.5 mg/g body weight on the 13th day of pregnancy. Hematoma in the region of the oral cavity (arrow).

Presumably, the external anomalies were associated with various developmental disorders of morphology and function of the internal organs.

Interestingly, subcutaneous hematomas often accompanied developmental anomalies such as ectrodactyly, syndactyly, brachymely, micrognathia and astomia (Fig. 10). These observations agree with those

Fig. 7.

Fig. 8.

Fig. 7. Control preparation of a 1-day mouse.

Fig. 8. Mouse fetus showing marked general deformation after injection of caffeine in the pregnant mouse in doses of 0.3 mg/g body weight on the 12th day of pregnancy.

Beginning on the ninth day after fertilization, numerous developmental disorders were observed in the progeny, the largest number being observed when caffeine was injected on the 12th and 13th days of pregnancy.

Developmental disorders of the extremities were observed after administration of the different doses of caffeine on the 9th, 10th, 11th, 12th, 13th and 14th days of pregnancy, but especially on the 12th and 13th days. It may be remarked in Table I that on these two days of pregnancy besides numerous extremity anomalies, developmental disorders were observed also in other organs, especially in the region of the oral cavity.

These observations suggest the conclusion that the 13th and 12th days of pregnancy are the critical period of organogenesis in the musculoskeletal system of the extremities and splanchnocranium in mice.

The experimental results also showed that the dose of 0.5 mg/g body weight of caffeine is on the boundary of tolerance by mice. After injection of this dose, 32% of the pregnant mice died. Doses of caffeine larger

reported by other investigators (1, 3). According to *Nishimura* and *Nakai*, developmental anomalies are connected with hematomas in developing organs; this is a direct effect of caffeine on the fetus, the placenta being unaffected. This hypothesis is supported by the results of studies on developmental disorders caused by deficiency of pantothenic acid (*Giroud* et al., and *Nelson* et al., cited after 3) and linoleic acid (*Martinet*, cited after 3). The cited authors observed subcutaneous hematomas in the neighborhood of developmental anomalies.

Fig. 11.

Fig. 12.

Fig. 11. One-day mouse after injection of caffeine in the pregnant mouse in doses of 0.4 mg/g body weight on the 11th day of pregnancy. Stump of the left anterior extremity.

Fig. 12. One-day mouse after injection of caffeine in the pregnant mouse in doses of 0.4 mg/g body weight on the 13th day of pregnancy. Curvature of the vertebral column.

Adoption of this interpretation of the mechanism of formation of developmental anomalies will require histologic investigation of the neighborhood of hematomas with coexisting developmental anomalies.

Compared with the doses of caffeine administered therapeutically in humans, the doses which induced developmental disorders in our experiments were disproportionately high.

REFERENCES

1. *Bertrand, M., Schwem, E. Frandon, R., Vague, A., Alary, J.*: C. R. Soc. Biol. (Paris), 1965, 159, 2189.
2. Materials of the Symposium on "Teratogenic effects of drugs". (In Polish). Bull. of the Institute of Drugs, Warsaw, 1965, 12, 4A.
3. *Nishimura, H., Nakai, K.*: Proc. Soc. Exp. Biol., 1960, 104, 140.
4. *Rugh, R.*: Experimental embryology. Burg. Publish. Comp., Minneapolis 1962.
5. *Woolam, D. H. M., Millen, J. W.*: Roy. Soc. Health J., 1961, 81, 165.

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ON THE QUESTION OF THE HEPATOTOXICITY OF CAFFEINE AND THEOPHYLLIN

by

O. Strubelt, F. Wegener and C.-P. Siegers

Dedicated to Professor Dr. F. Heim on his 60th birthday

Several experiments with animals and clinical investigations of recent years have shown that, after intake of caffeine and theophyllin, as well as caffeine-containing coffee, there is an increase in the free fatty acids of the serum (Bellet et al. 1965, 1968a, 1968b; Hynie et al. 1966; Stock and Westermann 1966; Triner and Nahas 1966; Zeller and Ammon 1967). This effect can be attributed to a mobilization of deposited fat as a consequence of activation of the triglyceride lipase of the fatty tissue (Stock and Westermann 1966). Cyclic 3',5'-adenosinemonophosphate is viewed as the mediator of the lipolytic effect of methylxanthine; as a result of the phosphotide esterases inhibition by theophyllin and caffeine, 3',5'-adenosinemonophosphate cannot be further decomposed (Butcher and Sutherland 1962; Hynie et al. 1966).

In the case of very marked lipolysis, the amount of free fatty acidds can exceed the requirements of the organs, so that only 10-15% is oxidized (Dole 1964); in this case, the greater part is resynthesized in the liver into triglycerides, transported back to the fat tissue in the form of lipoproteins and deposited there (Steinberg 1966). This yield of triglycerides from the liver to the serum is an active process, which can be disturbed by liver poisons such as carbon tetrachloride to such an extent that a fatty degeneration of the liver results (Recknagel 1967).

Estler and Ammon (1966) have determined that, after caffeine administration to mice, the concentration of esterified fatty acids in the liver increases. This finding makes it seem possible that methylxanthine, like carbon tetrachloride, inhibits the triglyceride secretion of the liver. Thus the question was raised as to whether there is a possible hepatotoxic effect of caffeine and theophyllin. This question is to be answered in this investigation.

Methodology

As experimental animals we used male Wistar rats (normal animals, Bäumler Animal Breedery) weighing 280-360 g. The animals were kept in groups of five at a temperature of 23°C and nourished with Altromin dry feed and water ad libitum. In order to avoid an unwanted activation of lipolysis as a result of hunger, we allowed the rats free access to food and water until we killed them.

The animals were anesthetized between 9 and 10 a.m. by means of injection of 1.2 g/kg urethane intraperitoneally, and their

blood drained a few minutes later through the carotid artery. Preliminary tests had shown that, in contrast to urethane anesthesia, ether anesthesia caused the level of free fatty acids in the serum to rise. We determined the concentration of glutamate-oxalacetate-transaminase (GOT) and glutamate-pyruvate-transaminase (GPT) in the serum in an optical test according to the method of Warburg, using combinations of tests of the firm of Boehringer, Mannheim; we determined the free fatty acids (FFA) according to the method of Duncombe (1964), the esterified fatty acids (EFA) according to the method of Fried and Hoeflmayr (1963) and the total cholesterine according to the method of Watson (1960) (test combinations of the firm of Haury). We determined the fat content of the liver in terms of total fatty acids (esterified and unesterified) according to the method of Van de Kamer et al. (1949).

The livers of all the animals that had received methylxanthine were examined histologically after fixation in formalin. Frozen and paraffin sections; stainings: hematoxylin-eosin, van Gieson, iron reaction according to Turnbull, fat staining with Sudan III. In addition, the heart, lungs, kidneys and spleen of most of the animals were examined using the same stainings; now and then the pancreas and adrenals were also included. Samples of daubs were taken from the control animals; the liver and often the other organs named were examined using the same method.

Caffeine and theophyllin were dissolved in a concentration of 1% in 0.9% saline solution. The controls received a corresponding amount of 0.9% saline solution. Since the concentrations of the esterified fatty acids and the total cholesterine in the serum varied considerably from day to day, it proved to be necessary to pair each test group with a control group examined on the same day.

In all the test groups, the mean values and their mean errors ($\bar{x} \pm \bar{x}$) were calculated in the conventional manner. For the comparison of two mean values we used t-distribution. A difference was considered significant if $P<0.05$.

Substances used: caffeinum anhydricum; theophyllinum anhydricum; analysis quality carbon tetrachloride (Merck); urethane (DAB 6).

Results

1. Caffeine and Theophyllin

Since in the experiments of Entler and Ammon (1966), the maximum increase in esterified fatty acids of the liver appeared 2 hours after administration of caffeine, we also selected a time interval of 2 hours for our experiments. Furthermore, we undertook our experiments 16 hours after administration of the methylxanthine, for after an interval of this length, hepatotoxic effects are in general more marked (Bruns and Wollenweber 1962; Strubelt et al. 1963). In order to become acquainted with the effect of a repeated dose of caffeine and theophyllin on the parameters examined, caffeine and theophyllin were also given 4 times at intervals of 24 hours; the animals in these experiments were killed 2 hours after the last dosage.

The results of our biochemical examinations are shown in Tables 1 and 2. 2 hours after intraperitoneal injection of

60 mg/kg caffeine or theophyllin we found an increase in the free fatty acids of 137 to 149% of the corresponding control values. (Table 1, nos. 1 and 5). In the case of four-day treatment with 60 mg/kg methylxanthine ip., the increase in free fatty acids 2 hours after the last injection was 87% of the control values for caffeine, 135% for theophyllin (nos. 3, 6). The subcutaneous administration of caffeine had an approximately identical effect on the free fatty acids as that of intraperitoneal injection (no.4). 16 hours after 60 mg/kg caffeine ip., an increase in the free fatty acids could no longer be determined (no. 2).

Tab. 1. The concentrations of free fatty acids (FFS), esterified fatty acids (VFS), cholesterine (total cholesterin), GOT and GPT in the serum, as well as the fat content of the liver (mg/g wet weight) after various pretreatments ($\bar{x} \pm s_{\bar{x}}$). T = time interval between the injections and the killing of the animals. N = number of experiments. *) = significant difference from the corresponding value of the corresponding control group ($P < 0.05$).

Nr.	A. Behandlung	B. Dosis/kg (ip.)	T (h)	N	FFS (µval/l)	VFS (mval/l)	Cholesterin (mg/100 ml)	SGOT (mU/ml)	SGPT (mU/ml)	C. Fettgehalt der Leber (mg/g)
1	a. Coffein	60 mg	2	8	433 ± 15*)	4,7 ± 0,9	113 ± 16	75,1 ± 6,6*)	18,4 ± 1,5*)	46 ± 1,4
	0,9%NaCl	6 ml	2	8	183 ± 36	5,5 ± 0,4	130 ± 26	58,4 ± 5,9	14,6 ± 0,9	38 ± 0,7
2	Coffein	60 mg	16	9	172 ± 13	4,5 ± 0,6*)	78 ± 17	114,0 ± 31,0	18,2 ± 2,0	45 ± 1,5
	0,9%NaCl	6 ml	16	7	144 ± 17	7,5 ± 1,0	97 ± 18	67,4 ± 6,0	15,8 ± 4,3	41 ± 8,1
3	Coffein	60 mg[1)]	2	12	282 ± 36*)	5,1 ± 0,2	108 ± 16	57,9 ± 4,0	15,0 ± 0,8	39 ± 1,0
	0,9%NaCl	6 ml[1)]	2	8	151 ± 22	5,8 ± 0,4	137 ± 20	53,0 ± 2,3	14,1 ± 0,7	37 ± 0,4
4	Coffein	60 mg[1)]	2	10	479 ± 14*)	7,3 ± 0,7	181 ± 11	58,1 ± 5,6	16,0 ± 1,0	40 ± 2,2
	0,9%NaCl	6 ml[1)]	2	10	230 ± 28	5,9 ± 0,4	186 ± 9	53,8 ± 1,4	15,1 ± 1,0	40 ± 1,3
5	Theophyllin	60 mg	2	11	542 ± 69*)	6,0 ± 0,5	139 ± 14	65,5 ± 2,8*)	12,6 ± 1,7	40 ± 1,1
	0,9%NaCl	6 ml	2	10	218 ± 19	5,3 ± 0,2	124 ± 11	53,7 ± 3,5	13,7 ± 1,2	32 ± 4,0
6	Theophyllin	60 mg[1)]	2	12	418 ± 52*)	4,9 ± 0,6	92 ± 16	53,0 ± 5,6	15,3 ± 1,2	44 ± 2,2
	0,9%NaCl	6 ml[1)]	2	11	178 ± 14	5,8 ± 0,7	119 ± 19	55,7 ± 2,7	16,3 ± 1,1	41 ± 2,4
7	Coffein	100 mg	2	10	450 ± 45*)	6,4 ± 0,6	266 ± 8	54,0 ± 3,7	10,4 ± 1,0	44 ± 1,0
	Theophyllin	100 mg	2	10	359 ± 37*)	6,9 ± 0,3	252 ± 8	62,6 ± 2,6	11,6 ± 0,9	44 ± 2,0
	0,9%NaCl	10 ml	2	10	153 ± 22	7,3 ± 0,5	260 ± 5	59,1 ± 2,9	10,5 ± 0,8	44 ± 1,0
8	Coffein	100 mg	16	10	412 ± 79*)	5,1 ± 0,4	234 ± 10*)	62,4 ± 7,8	13,9 ± 1,9	47 ± 3,0*)
	Theophyllin	100 mg	16	10	345 ± 37*)	6,2 ± 0,5	223 ± 12	59,7 ± 4,5	9,0 ± 0,8	47 ± 2,4*)
	0,9%NaCl	10 ml	16	10	209 ± 31	6,3 ± 0,5	179 ± 20	58,7 ± 3,3	11,6 ± 0,7	36 ± 1,0

[1)] Diese Tiere erhielten die gleiche Behandlung auch an den 3 vorangehenden Tagen.
[2)] Bei dieser Versuchsgruppe erfolgte die Injektion subcutan.

Key:
A. treatment B. dose/kg C. fat content of the liver
a. caffeine
1) These animals received the same treatment on the 3 preceding days as well.
2) Injection to this test group was subcutaneous.

We found an effect on the esterified fatty acids only in one group, namely 16 hours after 60 mg/kg caffeine ip. (no. 2); here the values were significantly lower than those of the comparison group. There were no alterations after administration

of 60 mg/kg methylxanthine in either the cholesterin content of the serum or the fat content of the liver.

Tab. 2. The concentrations of esterified fatty acids (VFS), GOT and GPT in the serum, as well as the fat content of the liver (mg/g wet weight) 24 hours after treatment with CCl_4 and caffeine, theophyllin or 0.9% NaCl ($\bar{x} \pm s_{\bar{x}}$). N = number of experiments.

A Behandlung	B Dosis/kg	N	VFS (mval/l)	SGOT (mU/ml)	SGPT (mU/ml)	C Fettgehalt der Leber (mg/g)
CCl_4 + Coffein	0,1 ml ip. 60 mg im.	9	4,1 ± 0,5	140 ± 18	70 ± 13	[illegible]
CCl_4 + Theophyllin	0,1 ml ip. 60 mg im.	9	5,6 ± 0,5	155 ± 15	71 ± 18	[illegible]
CCl_4 + 0,9% NaCl	0,1 ml ip. 6 ml im.	15	3,1 ± 0,4	100 ± 10	71 ± 9	[illegible]

For key see table 1.

The serum enzymes GOT and GPT were slightly, but significantly nonetheless, increased 2 hours after ip. injection of 60 mg/kg caffeine (no. 1). 2 hours after injection of 60 mg/kg theophyllin, there was a significant increase in the SGOT. These findings led us to perform a second test series with 100 mg/kg caffeine and theophyllin, whereby the examinations were performed 2 and 16 hours after administration of methylxanthine (table 1).

Caffeine and theophyllin in the higher dose increased the free fatty acids of the blood by 194 or 135% after 2 hours. In contrast to the results with 60 mg/kg, we saw in this case an increase of 97 or 65% in the free fatty acids after 16 hours. At this time, the fat content of the liver was also increased by 24%. Beyond this, 16 hours after 100 mg/kg caffeine, we found a slight increase in the cholesterin content in the blood. The esterified fatty acids and the serum enzymes were not affected, on the other hand, by 100 mg/kg caffeine or theophyllin.

A histological examination of groups 1-6 (table 1) revealed the same findings for the animals treated with caffeine or theophyllin and those treated with saline solution. We observed no differences between the groups either. About 1/3 of the animals of each group (treated and controls) manifested no liver alterations at all. In the other animals we found fatty degeneration of single cells distributed randomly over the hepatic lobules; these were mostly fine drops, only rarely large drops or fine to medium drops, and only rarely large drops and univacuolar. In sum, these single cell fatty degenerations could be designated as sparse; only in a few cases were they moderately marked. In single cases, a more intense fatty degeneration of the Kupffer cells was seen, without any determinable frequency in any one of the groups. A thorough examination of the hepatic cell nuclei revelaed no alterations. Double nuclei were found only rarely.

Very seldom we found typical hepatic cell mitoses. Cytoplasmic alterations were never observed. No cell decomposition pigment (ceroid) was found. No pathological alterations were determined in any of the other organs examined.

Among the animals treated with 100 mg/kg caffeine or theophyllin, those of group 7 (2 hours) also revealed the findings already described: single cell fatty degenerations of slight degree throughout the liver, more marked only rarely, some cases with fatty degeneration of the Kupffer cells, no alterations of other organs. 16 h after a dose of 100 mg/kg caffeine or theophyllin (group 8) we found, on the other hand, clearly more marked fatty degeneration of the liver. It was mostly of the single cell type, but we occasionally also saw fatty degenrations of markedly zonal arrangement, affecting the entire peripheral and intermediate lobule zones. Fatty degenerations of the Kupffer cells were observed in about half of the animals. Otherwise no pathological alterations could be determined in the liver cells. The controls of group 8 did not differ from the remaining controls in their histological aspect.

A secondary finding in all the theophyllin-treated animals of group 8 were intense alterations of the myocardium: in both ventricles we found scattered, partly very expanded lumpy acidophilic fiber group necroses of the myocardium, with mostly very considerable mesenchyma cell proliferations of predominantly histiocytaric nature. In 2 of the animals treated with caffeine, we observed sparse, very small myocardial necroses with circumscribed granuloma-like histiocyte proliferation.

2. Carbon Tetrachloride, Caffeine and Theophyllin

In order to clarify whether methylxanthine can be considered a potentiating factor of liver damage, we produced an experimental liver lesion with 0.1 ml/kg carbon tetrachloride intraperitoneally. At the same time the animals received 60 mg/kg caffeine or theophyllin or 6 ml/kg 0.9% NaCl solution im. 24 hours later, the animals were killed and the esterified fatty acids, GOT and GPT of the blood, as well as the fat content of the liver, were determined. The results of these experiments can be seen in Table 2. After administration of carbon tetrachloride, the esterified fatty acids in all 3 groups were lower than the values we had found in the case of the animals whose livers were not damaged (Tab. 1). A further manifestation of this liver lesion was an increase in the SGOT to about double and the SGPT to about four to five times the normal values. The fat content of the liver was also increased by carbon tetrachloride. However, we found no differences between the groups that had on the one hand received caffeine or theophyllin, and on the other 0.9% NaCl solution.

In the case of all the animals shown in Table 2, the livers manifested identical alterations. In the central lobule zones we found marked hydropic degeneration of the hepatic cells, partly with karyolysis. There were scattered acidophilic single cell necroses. At the same time, there was an intensive histiocyte proliferation in the lobule centers. Around the altered lobule center there were always found varyingly wide zones with markedly fine-drop fat degenerated hepatic cells. The extent of the de-

degenerative and necrotic processes, as well as the fatty degeneration did not differ in the case of additional caffeine or theophyllin administration from the findings in the case of pure carbon tetrachloride poisoning.

Discussion

In the experiments of Estler and Ammon (1966), which were conducted on mice, the concentration of free fatty acids in the serum increased 2 hours after administration of 50 mg/kg caffeine by about 150%. At the same time, in the liver, there was an increase of about 55% in the esterified fatty acids. In our experiments with rats, the free fatty acids of the serum also increased about 1½ times after 60 mg/kg caffeine or theophyllin. However, in contrast to Estler and Ammon, we were not able to determine changes in the fat content of the liver after these doses of methylxanthine.

This different result could not be a consequence of different methodology. Estler and Ammon did determine the concentration of the esterified fatty acids in the liver; we, on the other hand, determined the total fatty acids, i.e. the esterified and unesterified fatty acids. Since, however, the concentration of free fatty acids in the liver is only about 5% of the concentration of the esterified fatty acids (Ugazio and Lombardi 1965), our findings can definitely be compared with the results of Estler and Ammon. This also results from the fact that the absolute quantity of the fatty acids determined in the liver was approximately the same in both examinations (about 40 mg/g wet weight).

Thus we can assume that it is a matter in this case of type-specific differences. Obviously the rat liver can better compensate for an increased amount of fat, by means of oxidation or triglyceride secretion, than can the mouse liver. However, it is a matter not of a qualitative, but only of a quantitative difference. Thus, as we were able to determine chemically and histologically, the higher dose of 100 mg/kg caffeine or theophyllin increased the fat content of the liver in the case of the rats as well. A long-lasting lipolysis -- the free fatty acids of the blood were still increased after 16 hours in these experiments -- can thus exceed the fat processing capacity of the liver of rats as well.

In order to clarify whether the increase in liver fat content produced by methylxanthine is the manifestation of a hepatotoxic effect, we examined the blood enzymes GOT and GPT. These enzymes are quite a sensitive indicator of hepatic lesions in rats as well (Strubelt et al. 1967). Indeed, we found, in 2 test series with 60 mg/kg caffeine or theophyllin, a slight increase in activity of these enzymes. In the remaining groups, which had received the same dose of caffeine or theophyllin, we did not, however, see any such increase in activity, especially not in the case of the animals which, besides the acute application, had also gone through a 3-day preliminary treatment with 60 mg/kg methylxanthine. Above all, however, the higher dose of 100 mg/kg caffeine and theophyllin did not affect the SGOT and SGPT; in the case of hepatotoxic substances such as carbon tetrachloride and allyl alcohol, on the other hand, there is a clear relation between

the dose and the extent of the enzyme increase (Bruns and Wollenweber 1962; Staubelt et al. 1967).

We are therefore of the opinion that, because of lack of reproducibility and dependence upon dosage, the enzyme increases we observed cannot be viewed as valid proof of a hepatotoxic effect of methylxanthine. Our histological findings, which offered no clue of any hepatic cell lesions in any of the animals treated with caffeine and theophyllin, also deny such an effect.

Recently, Schwetz and Plaa (1969) observed that adrenaline and noradrenaline strengthen the hepatotoxic activity of carbon tetrachloride. Since the methylxanthines release the catecholamines of the organism (Atuk et al. 1967; DeSchaepdryver 1959; Levi 1967), it seemed plausible that they also strengthen the CCl_4 hepatic lesions. However, this was not the case -- probably because the catecholamine quantities released by caffeine or theophyllin were considerably lower than those introduced exogenically by Schwetz and Plaa (1969).

Summary

1. 2 and 16 hours after administration of caffeine and theophyllin (60 and 100 mg/kg ip.), the concentrations of free fatty acids, esterified fatty acids, total cholesterine, SGOT and SGPT in the blood of rats, as well as the fat content and the histological aspect of the liver were examined.

Both methylxanthines increased the free fatty acids of the serum by double or triple the initial amount. In the case of the 60 mg/kg dose, this effect was observed only after 2 hours; in the case of the 100 mg/kg dose, it was still present after 16 hours. The concentrations of the esterified fatty acids and the cholesterine of the serum were not notably affected by caffeine or theophyllin. 2 hours after administration of 60 mg/kg caffeine and theophyllin, the SGOT and SGPT were slightly increased; this enzyme increase proved, however, to be neither reproducible nor dose-dependent.

The fat content of the liver was raised only by 100 mg/kg caffeine and theophyllin after 16 hours, by 24%. In the case of these animals, we also found, histologically, fatty degeneration of an intense degree. In none of the animals treated with caffeine or theophyllin was there histologically found any sign of hepatic cell damage.

2. 24 hours after administration of 0.1 ml/kg carbon tetrachloride ip., the SGOT and SGPT, as well as the fat content of the liver, were increased, while the concentration of the esterified fatty acids in the serum was lowered. Histologically, we found hepatic cell damage typical of a CCl_4 poisoning. The additional administration of caffeine and theophyllin (60 mg/kg im.) had no effect on the alterations caused by CCl_4.

3. Since we found no definite evidence of a hepatotoxic activity of the methylxanthines, the rise in liver fat content caused by caffeine and theophyllin in larger doses cannot be viewed as the manifestation of hepatic lesion, but only as the result of a lipolysis exceeding the fat metabolism capacity of the liver.

4. 16 hours after administration of 100 mg/kg theophyllin, we found myocardial necroses of severe degree in all the animals.

Following the same dose of caffeine, slighter necroses of the myocardium were determined in 2 out of 10 animals.

Literature

Atuk, N. O., Blaydes, M. C., Westervelt, F. B. u. Wood, J. E., Circulation 35, 745 (1967)

Bellet, S., Kershbaum, A. u. Aspe, J., Arch. Intern. Med. 116, 750 (1963)

Bellet, S., Kershbaum, A. u. Flack, E. M., Metabolism 17, 702 (1968a)

Bellet, S., Feinberg, L. J., Sandberg, H. u. Hirabayashi, M., J. Pharmacol. exp. Ther. 159, 250 (1968b)

Bruns, F. H. u. Wollenweber, J., Klin. Wschr. 40, 993 (1962)

Butcher, R. W. u. Sutherland, E. W., J. biol. Chem. 237, 1244 (1962)

De Schaepdryver, A. F., Arch. int. Pharmacodyn. 119, 517 (1959)

Dole, V. P., Fat as a tissue, Ed. K. Rodahl, McGraw-Hill, New York, 1964, S. 250

Duncombe, W. G., Clin. chim. Acta 9, 122 (1964)

Estler, C.-J. u. Ammon, H. P. T., Experientia (Basel) 22, 909 (1966)

Fried, R. u. Hoeflmayr, J., Klin. Wschr. 41, 727 (1963)

Hynie, S., Krishna, G. u. Brodie, B. B., J. Pharmacol. exp. Ther. 153, 90 (1966)

Levi, L., Acta med. scand. 181, 431 (1967)

Reckenagel, R. O., Pharmacol. Rev. 19, 145 (1967)

Schwetz, B. A. u. Plaa, G. L., Toxicol. appl. Pharmacol. 14, 495 (1969)

Steinberg, D., Pharmacol. Rev. 18, 217 (1966)

Stock, K. u. Westermann, E., Naunyn-Schmiedeberg's Arch. Pharmak. exp. Path. 254, 334 (1966)

Strubelt, O., Tillig, K. u. Wolf, F., Med. exp. (Basel) 9, 341 (1963)

Strubelt, O., Breining, H. u. Wüst, H., Arch. Toxikol. 22, 236 (1967)

Trinor, L. u. Nahas, G. G., J. Pharmacol. exp. Ther. 151, 969 (1966)

Ugazio, G. u. Lombardi, B., Lab. Invest. 14, 711 (1965)

Van de Kamer, J. H., ten Bokkel, H. u. Weijers, H. A., J. biol. Chem. 177, 347 (1949)

Watson, D., Clin. chim. Acta 5, 637 (1960)

Zeller, W. u. Ammon, H. P. T., Z. Gastroenterol. 5, 84 (1967)

Strubelt-'70
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Aus dem Institut für Pharmakologie (Direktor: Professor Dr. G. Zetler)
und dem Pathologischen Institut (Direktor: Professor Dr. E. Jeckeln)
der Medizinischen Akademie Lübeck

Zur Frage der Hepatotoxizität von Coffein und Theophyllin

Herrn Professor Dr. F. Heim zum 60. Geburtstag gewidmet

Von O. Strubelt, F. Wegener und C.-P. Siegers

Mehrere tierexperimentelle und klinische Untersuchungen der letzten Jahre haben gezeigt, daß es nach Applikation von Coffein und Theophyllin sowie auch nach coffeinhaltigem Kaffee zu einem Anstieg der freien Fettsäuren des Serums kommt (Bellet et al. 1968a, 1968b; Hynie et al. 1966; Stock u. Westermann 1966; Trinc u. Nahas 1966; Zeller u. Ammon 1967). Dieser Effekt ist auf eine Mobilisierung von Depotfett infolge Aktivierung der Triglyceridlipase des Fettgewebes zurückzuführen (Stock u. Westermann 1966). Als Mediator der lipolytischen Wirkung der Methylxanthine wird das cyclische 3',5'-Adenosinmonophosphat angesehen, das infolge der durch Theophyllin und Coffein gehemmten Phosphodiesterase nicht mehr abgebaut werden kann (Butcher u. Sutherland 1962; Hynie et al. 1966).

Bei sehr starker Lipolyse kann das Angebot an freien Fettsäuren den Bedarf der Organe übersteigen, so daß nur 10—15% oxydiert werden (Dole 1964); der größere Teil wird in diesem Fall in der Leber zu Triglyceriden resynthetisiert, in Form von Lipoprotein in die Fettgewebe rücktransportiert und hier abgelagert (Steinberg 1966). Diese Abgabe von Triglyceriden aus der Leber an das Serum ist ein aktiver Prozeß, der durch Lebergifte wie Tetrachlorkohlenstoff soweit gestört werden kann, daß eine Leberverfettung resultiert (Recknagel 1967).

Estler u. Ammon (1966) haben nun nachgewiesen, daß nach Coffein bei Mäusen die Konzentration veresterter Fettsäuren in der Leber ansteigt. Dieser Befund läßt es möglich erscheinen, daß die Methylxanthine wie Tetrachlorkohlenstoff die Triglyceride-Sekretion der Leber hemmen. Es erhob sich somit die Frage nach einer möglichen hepatotoxischen Wirkung von Coffein und Theophyllin, die in der vorliegenden Untersuchung beantwortet werden soll.

Methodik

Als Versuchstiere dienten männliche Wistar-Ratten (konventionelle Tiere, Tierzüchterei Bäumler) im Gewicht von 230—360 g. Die Tiere wurden bei einer Temperatur von 23° C in Gruppen zu 5 gehalten und mit Altromin®-Trockenfutter und Wasser ad libitum ernährt. Um eine unerwünschte Aktivierung der Lipolyse infolge Hungers zu vermeiden, beließen wir den Tieren bis zur Tötung freien Zugang zu Futter und Wasser.

Die Tiere wurden zwischen 9 und 10 Uhr vormittags durch Injektion von 1,2 g/kg Urethan ip. narkotisiert und wenige Minuten später durch Eröffnen der A. carotis entblutet. Vorversuche hatten gezeigt, daß eine Äthernarkose im Unterschied zum Urethan den Spiegel der freien Fettsäuren im Serum ansteigen läßt. Wir bestimmten die Konzentration der Glutamat-Oxalacetat-Transaminase (GOT) und Glutamat-Pyruvat-Transaminase (GPT) im Serum im optischen Test nach Warburg unter Verwendung von Testkombinationen der Firma Boehringer, Mannheim, die freien Fettsäuren (FFS) nach Duncombe (1964), die veresterten Fettsäuren (VFS) nach Fried u. Hoeflmayr (1963) und Gesamt-Cholesterin nach Watson (1960) (Testkombinationen der Firma Haury). Den Fettgehalt der Leber ermittelten wir als Gesamt-Fettsäuren (veresterte und unveresterte) nach der Methode von Van de Kamer et al. (1949).

Die Lebern aller Tiere, die Methylxanthine erhalten hatten, wurden nach Formalinfixierung histologisch untersucht. Gefrier- und Paraffinschnitte; Färbungen: Haematoxylin-Eosin, van Gieson, Eisenreaktion nach Turnbull, Fettfärbung mit Sudan III. Dazu wurden von den meisten Tieren unter Anwendung der gleichen Färbungen Herz, Lunge, Niere und Milz untersucht, hin und wieder auch Pankreas und Nebennieren. Von den Kontrolltieren wurden Stichproben entnommen, dabei stets die Leber und meist auch die übrigen genannten Organe nach gleicher Methode untersucht.

Coffein und Theophyllin wurden in einer Konzentration von 1% in 0,9%iger Kochsalzlösung gelöst. Die Kontrolltiere erhielten eine entsprechende Menge 0,9%iger Kochsalzlösung. Da die Konzentrationen der veresterten Fettsäuren und des Gesamt-Cholesterins im Serum von Tag zu Tag erheblich schwankten, erwies es sich als notwendig, jeder Versuchsgruppe eine eigene, am gleichen Tag untersuchte Kontrollgruppe zuzuordnen.

In allen Versuchsgruppen wurden die Mittelwerte und ihre mittleren Fehler ($\bar{x} \pm \bar{x}$) in üblicher Weise berechnet. Zum Vergleich zweier Mittelwerte benutzten wir die t-Verteilung. Ein Unterschied wurde als signifikant angesehen, wenn $P < 0{,}05$ war.

Verwendete Substanzen: Coffeinum anhydricum; Theophyllinum anhydricum; Tetrachlorkohlenstoff Analysenqualität (Merck); Urethan (DAB 6).

Versuchsergebnisse

1. Coffein und Theophyllin

Da in den Untersuchungen von Estler u. Ammon (1966) das Maximum im Anstieg der veresterten Fettsäuren der Leber 2 h nach Applikation von Coffein aufgetreten war, wählten wir für unsere Versuche ebenfalls einen Zeitabstand von 2 h. Wir nahmen unsere Untersuchungen außerdem 16 h nach Applikation der Methylxanthine vor, weil nach einem derart längeren Zeitraum hepatotoxische Wirkungen im allgemeinen stärker ausgeprägt sind (Bruns u. Wollenweber 1962; Strubelt et al. 1963). Um den Einfluß einer wiederholten Gabe von Coffein und Theophyllin auf die untersuchten Parameter kennenzulernen, wurden Coffein und Theophyllin auch 4mal im Abstand von 24 h gegeben; die Tiere wurden in diesen Versuchen 2 h nach der letzten Applikation getötet.

Die Ergebnisse unserer biochemischen Untersuchungen sind in Tab. 1 und 2 zusammengestellt. 2 h nach 60 mg/kg Coffein bzw. Theophyllin ip. fanden wir einen Anstieg der freien Fettsäuren um 137 bzw. 149% der entsprechenden Kontrollwerte (Tab. 1, Nr. 1 und 5). Bei viertägiger Behandlung mit 60 mg/kg der Methylxanthine ip. betrug der Anstieg der freien Fettsäuren 2 h nach der letzten Injektion bei Coffein 87%, bei Theophyllin dagegen 135% der Kontrollwerte (Nr. 3, 6). Die subcutane Applikation von Coffein hatte einen annähernd gleichen Effekt auf die freien Fettsäuren wie die intraperitoneale (Nr. 4). 16 h nach 60 mg/kg Coffein ip. ließ sich ein Anstieg der freien Fettsäuren nicht mehr nachweisen (Nr. 2).

Eine Beeinflussung der veresterten Fettsäuren fanden wir nur in einer Gruppe, nämlich 16 h nach 60 mg/kg Coffein ip. (Nr. 2); hier waren die Werte signifikant niedriger als die der Vergleichsgruppe. Im Cholesteringehalt des Serums sowie im Fettgehalt der Leber traten nach 60 mg/kg der Methylxanthine keine Änderungen ein.

Die Serumfermente GOT und GPT waren 2 h nach 60 mg/kg Coffein ip. geringgradig, aber doch signifikant erhöht (Nr. 1). 2 h nach 60 mg/kg Theophyllin fand sich ein signifikanter Anstieg der SGOT. Diese Befunde veranlaßten uns, eine 2. Versuchsserie mit 100 mg/kg Coffein und Theophyllin durchzuführen, wobei die Untersuchungen 2 und 16 h nach Applikation der Methylxanthine erfolgten (Tab. 1).

Coffein und Theophyllin steigerten in der höheren Dosierung die freien Fettsäuren des Serums nach 2 h um 194 bzw. 155%. Im Unterschied zu den Ergebnissen mit 60 mg/kg sahen wir jetzt auch noch nach 16 h eine Erhöhung der freien Fettsäuren um 97 bzw. 65%. Zu diesem Zeitpunkt war auch der Fettgehalt der Leber um 24% erhöht. Außerdem fand sich 16 h nach 100 mg/kg Coffein eine leichte Steigerung des Cholesteringehaltes im Serum. Die veresterten Fettsäuren und die Serumfermente wurden dagegen durch 100 mg/kg Coffein bzw. Theophyllin nicht beeinflußt.

Bei der histologischen Durchmusterung der Gruppen 1—6 (Tab. 1) ergaben sich bei den mit Coffein bzw. Theophyllin einerseits und Kochsalzlösung andererseits behandelten Tieren gleiche Befunde. Auch zwischen den Gruppen beobachteten wir keine Unterschiede. Etwa 1/3 der Tiere jeder Gruppe (behandelte wie Kontrolltiere) zeigte überhaupt keine Leberveränderungen. Bei den übrigen Tieren fanden sich ohne

Tab. 1: Die Konzentrationen an freien Fettsäuren (FFS), veresterten Fettsäuren (VFS), Cholesterin (Gesamt-Cholesterin), GOT und GPT im Serum sowie der Fettgehalt der Leber (mg/g Feuchtgewicht) nach unterschiedlicher Vorbehandlung ($\bar{x} \pm s_{\bar{x}}$). T = Zeitraum zwischen den Injektionen und der Tötung der Tiere. N = Zahl der Versuche. *) = signifikanter Unterschied zum entsprechenden Wert der jeweiligen Kontrollgruppe ($P < 0{,}05$).

Nr	Behandlung	Dosis/kg (ip.)	T (h)	N	FFS (µval/l)	VFS (mval/l)	Cholesterin (mg/100 ml)	SGOT (mU/ml)	SGPT (mU/ml)	Fettgehalt der Leber (mg/g)
1	Coffein	60 mg	2	8	433 ± 15*)	4,7 ± 0,9	113 ± 16	75,1 ± 6,6*)	18,4 ± 1,5*)	61 ± 1,6
	0,9%NaCl	6 ml	2	8	183 ± 36	5,5 ± 0,4	130 ± 26	58,4 ± 3,9	14,6 ± 0,9	59 ± 0,7
2	Coffein	60 mg	16	9	179 ± 13	4,5 ± 0,6*)	78 ± 17	114,0 ± 31,0	18,3 ± 0,0	61 ± 1,3
	0,9%NaCl	6 ml	16	7	144 ± 17	7,5 ± 1,0	97 ± 18	67,4 ± 6,0	15,1 ± 4,3	61 ± 0,1
3	Coffein	60 mg[1)]	2	12	282 ± 36*)	5,1 ± 0,2	108 ± 16	57,9 ± 4,0	15,0 ± 0,6	59 ± 1,0
	0,9%NaCl	6 ml[1)]	2	8	151 ± 22	5,8 ± 0,4	137 ± 20	53,9 ± 2,3	14,1 ± 0,3	57 ± 0,8
4	Coffein	60 mg[2)]	2	10	479 ± 14*)	7,3 ± 0,7	181 ± 11	58,1 ± 5,6	16,0 ± 1,0	60 ± 2,2
	0,9%NaCl	6 ml[1)]	2	10	230 ± 28	5,9 ± 0,4	186 ± 9	53,8 ± 1,4	15,1 ± 1,0	59 ± 1,3
5	Theophyllin	60 mg	2	11	542 ± 69*)	6,0 ± 0,5	138 ± 14	65,5 ± 2,8*)	12,6 ± 1,7	60 ± 1,3
	0,9%NaCl	6 ml	2	10	218 ± 19	5,3 ± 0,2	124 ± 1	53,7 ± 3,5	15,7 ± 1,2	55 ± 4,0
6	Theophyllin	60 mg[1)]	2	12	418 ± 52*)	4,9 ± 0,6	92 ± 16	53,0 ± 5,4	13,3 ± 1,3	61 ± 2,2
	0,9%NaCl	6 ml[1)]	2	11	178 ± 14	5,8 ± 0,7	119 ± 19	55,7 ± 2,7	14,3 ± 1,1	61 ± 2,9
7	Coffein	100 mg	2	10	450 ± 45*)	6,4 ± 0,6	266 ± 8	54,0 ± 3,7	10,4 ± 1,0	44 ± 1,0
	Theophyllin	100 mg	2	10	359 ± 37*)	6,9 ± 0,3	252 ± 8	62,6 ± 2,8	11,4 ± 0,9	44 ± 2,0
	0,9%NaCl	10 ml	2	10	153 ± 22	7,3 ± 0,5	260 ± 5	59,1 ± 2,9	10,3 ± 0,9	44 ± 1,0
8	Coffein	100 mg	16	10	412 ± 79*)	5,1 ± 0,4	234 ± 10*)	62,4 ± 7,8	13,9 ± 1,9	47 ± 3,0*)
	Theophyllin	100 mg	16	10	345 ± 37*)	6,2 ± 0,5	223 ± 12	59,7 ± 4,5	9,0 ± 0,8	47 ± 2,4*)
	0,9%NaCl	10 ml	16	10	209 ± 31	6,3 ± 0,5	179 ± 20	56,7 ± 3,3	11,6 ± 0,7	53 ± 1,0

[1)] Diese Tiere erhielten die gleiche Behandlung auch an den 3 vorangehenden Tagen.
[2)] Bei dieser Versuchsgruppe erfolgte die Injektion subcutan.

Tab. 2: Die Konzentrationen an veresterten Fettsäuren (VFS), GOT und GPT im Serum sowie der Fettgehalt der Leber (mg/g Feuchtgewicht) 24 h nach Behandlung mit CCl_4 und Coffein, Theophyllin bzw. 0,9% NaCl ($\bar{x} \pm s_{\bar{x}}$). N = Zahl der Versuche.

Behandlung	Dosis/kg	N	VFS (mval/l)	SGOT (mU/ml)	SGPT (mU/ml)	Fettgehalt der Leber (mg/g)
CCl_4 + Coffein	0,1 ml ip. 60 mg im.	9	4,1 ± 0,5	140 ± 14	70 ± 13	58 ± 0,2
CCl_4 + Theophyllin	0,1 ml ip. 60 mg im.	9	3,6 ± 0,3	135 ± 15	71 ± 12	62 ± 3,9
CCl_4 + 0,9% NaCl	0,1 ml ip. 6 ml im.	10	4,1 ± 0,4	120 ± 10	71 ± 9	57 ± 3,4

Ordnung über die Leberläppchen verteilte Einzelzellverfettungen, meist feintropfig, nur selten grobtropfig oder fein- bis mittelgrobtropfig, nur einen großtropfig und univacuolär. In gesamt waren diese Einzelzellverfettungen als spärlich, nur in wenigen Fällen als mittelstark zu bezeichnen. Vereinzelt wurde auch eine stärkere Verfettung der Kupfer'schen Sternzellen gesehen. Es erkrankten Häufung in einer der Gruppen. Die einzelnen Veränderungen der Leberzellkerne hielt niemals Veränderungen erkennen. Doppelkernigkeit wurde selten angetroffen. Ganz vereinzelt fanden sich typische Leberzellmi-

losen. Cytoplasmaveränderungen wurden nirgends beobachtet. Es fand sich kein Zellzerfallspigment (Ceroid). Auch an den weiteren untersuchten Organen ließen sich keine pathologischen Veränderungen feststellen.

Unter den mit 100 mg/kg Coffein bzw. Theophyllin behandelten Tieren zeigten die der Gruppe 7 (2 h) auch nur die bereits geschilderten Befunde: an der Leber durchweg Einzelzellverfettungen leichten Grades, nur manchmal etwas stärkeren Ausmaßes, einige Fälle mit Verfettung der Kupffer'schen Sternzellen, keine Veränderungen anderer Organe. 16 h nach Gabe von 100 mg/kg Coffein bzw. Theophyllin (Gruppe 8) fanden wir dagegen eine deutlich stärkere Verfettung der Leber. Sie zeigte meist den Einzelzelltyp, doch sahen wir gelegentlich auch Verfettungen in ausgeprägt zonaler Anordnung, die die gesamten peripheren und intermediären Läppchenzonen betrafen. Verfettungen der Kupffer'schen Sternzellen sahen wir bei etwa der Hälfte der Tiere. Sonst waren an den Leberzellen keine pathologischen Veränderungen festzustellen. Die Kontrollen der Gruppe 8 unterschieden sich in ihrem histologischen Bild nicht von den übrigen Kontrollen.

Als Nebenbefund zeigten alle mit Theophyllin behandelten Tiere der Gruppe 8 schwere Herzmuskelveränderungen: In beiden Ventrikeln fanden sich verstreute, z. T. sehr ausgedehnte schollige acidophile Faszergruppennekrosen des Myocards mit meist sehr beträchtlichen Mesenchymzellproliferationen vorwiegend histiocytären Charakters. Bei 2 der mit Coffein behandelten Tiere dieser Gruppe sahen wir spärliche kleinste Herzmuskelnekrosen mit umschriebener granulomartiger Histiocytenwucherung.

Tetrachlorkohlenstoff, Coffein und Theophyllin

Um zu klären, ob die Methylxanthine als ein potenzierender Faktor von Leberschädigungen in Frage kommen, riefen wir eine experimentelle Leberschädigung durch 0,1 ml/kg Tetrachlorkohlenstoff ip. hervor. Gleichzeitig erhielten die Tiere 60 mg/kg Coffein bzw. Theophyllin oder 6 ml/kg 0,9%iger NaCl-Lösung im. 24 h später wurden die Tiere getötet und im Serum die veresterten Fettsäuren, GOT und GPT sowie der Fettgehalt der Leber bestimmt. Die Ergebnisse dieser Versuche sind aus Tab. 2 zu ersehen. Die veresterten Fettsäuren des Serums lagen nach Gabe von Tetrachlorkohlenstoff in allen 3 Gruppen niedriger als die Werte, die wir an nicht lebergeschädigten Tieren gefunden hatten (Tab. 1). Als Ausdruck der Leberschädigung fanden wir außerdem einen Anstieg der SGOT auf etwa das Doppelte und der SGPT auf das Vier- bis Fünffache der Normalwerte. Auch der Fettgehalt der Leber wurde durch Tetrachlorkohlenstoff erhöht. Unterschiede zwischen den Gruppen, die einerseits Coffein bzw. Theophyllin und andererseits 0,9%ige NaCl-Lösung erhalten hatten, fanden sich jedoch nicht.

Bei allen in Tab. 2 aufgeführten Tieren zeigten die Lebern übereinstimmende Veränderungen: Es fanden sich in den zentralen Läppchenzonen sehr ausgeprägte hydropische Degenerationen der Leberzellen, z. T. mit Karyolyse. Eingestreut waren acidophile Einzelzellnekrosen. Gleichzeitig bestand eine stärkere Histiocytenproliferation in den Läppchenzentren. Um das veränderte Läppchenzentrum fand sich stets eine wechselnd breite Zone mit stark feintropfig verfetteten Leberzellen. Das Ausmaß der degenerativen und nekrotisierenden Prozesse sowie der Verfettung unterschied sich bei zusätzlicher Coffein- oder Theophyllin-Gabe nicht von den Befunden bei der reinen Tetrachlorkohlenstoffvergiftung.

Diskussion

In den Versuchen von Estler u. Ammon (1966), die an Mäusen unternommen wurden, erhöhte sich die Konzentration der freien Fettsäuren im Serum 2 h nach Gabe von 50 mg/kg Coffein um etwa 150%. Gleichzeitig kam es in der Leber zu einer Zunahme der veresterten Fettsäuren um etwa 55%. In unseren Versuchen an Ratten stiegen die freien Fettsäuren des Serums nach 60 mg/kg Coffein oder Theophyllin ebenfalls um etwa das 1½fache an. Änderungen im Fettgehalt der Leber konnten wir jedoch nach diesen Dosen der Methylxanthine im Unterschied zu Estler u. Ammon nicht feststellen.

Dieses unterschiedliche Ergebnis dürfte nicht eine Folge unterschiedlicher Methodik sein. Zwar bestimmten Estler u. Ammon in der Leber die Konzentration der veresterten Fettsäuren, wir dagegen die Gesamtfettsäuren, also veresterte und unveresterte Fettsäuren; da aber die Konzentration der freien Fettsäuren in der Leber nur etwa 5% der Konzentration der veresterten Fettsäuren beträgt (Ugazio u. Lombardi 1965), können unsere Befunde mit den Ergebnissen von Estler u. Ammon durchaus verglichen werden. Dies ergibt sich auch daraus, daß die absolute Menge der in der Leber bestimmten Fettsäuren in beiden Untersuchungen annähernd gleich groß war (um 40 mg/g Feuchtgewicht).

Es ist also anzunehmen, daß es sich hier um artspezifische Unterschiede handelt. Offenbar kann die Leber der Ratte durch Oxydation oder Triglycerid-Sekretion ein vermehrtes Angebot von Fett besser kompensieren als die der Maus. Hierbei handelt es sich aber nicht um einen qualitativen, sondern nur um einen quantitativen Unterschied. Die höhere Dosis von 100 mg/kg Coffein oder Theophyllin erhöhte nämlich, wie sich chemisch und histologisch nachweisen ließ, auch bei der Ratte den Fettgehalt der Leber. Eine langdauernde Lipolyse — die freien Fettsäuren des Serums waren in diesen Versuchen noch 16 h später erhöht — kann also auch bei der Ratte die Fettverarbeitungskapazität der Leber übersteigen.

Um zu klären, ob der durch die Methylxanthine ausgelöste Anstieg des Leberfettgehaltes Ausdruck einer hepatotoxischen Wirkung ist, untersuchten wir die Serumfermente GOT und GPT. Diese Fermente sind auch bei der Ratte ein recht empfindlicher Indikator für Leberschädigungen (Strubelt et al. 1967). Tatsächlich fanden wir in 2 Versuchsserien mit 60 mg/kg Coffein bzw. Theophyllin eine geringe Aktivitätssteigerung dieser Fermente. In den übrigen Gruppen, die die gleiche Dosis von Coffein bzw. Theophyllin erhalten hatten, sahen wir jedoch keine derartigen Aktivitätssteigerungen, insbesondere nicht bei den Tieren, die neben der akuten Applikation auch noch eine 3tägige Vorbehandlung mit 60 mg/kg der Methylxanthine durchgemacht hatten. Vor allem aber ließ die höhere Dosis von 100 mg/kg Coffein und Theophyllin die SGOT und SGPT unbeeinflußt; bei hepatotoxischen Substanzen wie Tetrachlorkohlenstoff und Allylalkohol besteht dagegen eine eindeutige Relation zwischen der Dosis und dem Ausmaß des Fermentanstiegs (Bruns u. Wollenweber 1962; Strubelt et al. 1967).

Wir sind deshalb der Ansicht, daß man die von uns beobachteten Fermentsteigerungen wegen fehlender Reproduzierbarkeit und Dosisabhängigkeit nicht als stichhaltigen Beweis für eine hepatotoxische Wirkung der Methylxanthine ansehen kann. Gegen eine solche Wirkung sprechen auch unsere histologischen Befunde, die bei keinem der mit Coffein und Theophyllin behandelten Tiere einen Anhalt für eine Leberzellschädigung ergaben.

Schwetz u. Plaa (1969) haben kürzlich nachgewiesen, daß Adrenalin und Noradrenalin die hepatotoxische Wirksamkeit von Tetrachlorkohlenstoff verstärken. Da die Methylxanthine körpereigene Katecholamine freisetzen (Atuk et al. 1967; De Schaepdryver 1959; Levi 1967), erschien es denkbar, daß sie die CCl_4-Leberschädigung ebenfalls potenzieren. Dies war jedoch nicht der Fall — wahrscheinlich deshalb, weil die durch Coffein bzw. Theophyllin freigesetzten Katecholaminmengen erheblich niedriger waren als die von Schwetz u. Plaa (1969) exogen zugeführten.

Zusammenfassung

1, 2 und 16 h nach Gabe von Coffein und Theophyllin (60 und 100 mg/kg ip.) wurden bei Ratten die Konzentrationen an freien Fettsäuren, veresterten Fettsäuren, Gesamtcholesterin, SGOT und SGPT im Serum sowie der Fettgehalt und das histologische Bild der Leber untersucht.

Beide Methylxanthine erhöhten die freien Fettsäuren des Serums auf das Doppelte bis Dreifache; bei der Dosis von 60 mg/kg war dieser Effekt nur nach 2 h, bei der Dosis von 100 mg/kg auch noch nach 16 h vorhanden. Die Konzentrationen der veresterten Fettsäuren und des Cholesterins im Serum wurden durch Coffein und Theophyllin nicht nennenswert beeinflußt.

SGOT und SGPT waren 2 h nach Gabe von 60 mg/kg Coffein und Theophyllin geringgradig erhöht; dieser Fermentanstieg erwies sich jedoch weder als dosisabhängig noch als reproduzierbar.

Der Fettgehalt der Leber wurde nur durch 100 mg/kg Coffein und Theophyllin nach 16 h um 24% erhöht. Bei diesen Tieren zeigten sich auch histologisch Verfettungen stärkeren Grades. Bei keinem der mit Coffein bzw. Theophyllin behandelten Tiere fanden sich histologisch Zeichen einer Leberzellschädigung.

2. 24 h nach Gabe von 0,1 ml/kg Tetrachlorkohlenstoff ip. waren SGOT und SGPT sowie der Fettgehalt der Leber erhöht, die Konzentration der veresterten Fettsäuren im Serum dagegen erniedrigt. Histologisch fanden sich die für eine CCl_4-Vergiftung typischen Leberzellschädigungen. Die zusätzliche Gabe von Coffein oder Theophyllin (60 mg/kg im.) hatte keinen Einfluß auf die durch CCl_4 ausgelösten Veränderungen.

3. Da sich kein sicherer Anhalt für eine hepatotoxische Wirksamkeit der Methylxanthine fand, ist die durch Coffein und Theophyllin in hoher Dosierung hervorgerufene Zunahme des Leberfettgehaltes nicht als Ausdruck einer Leberschädigung, sondern nur als Folge einer die Fettverarbeitungskapazität der Leber übersteigenden Lipolyse anzusehen.

4. 16 h nach Gabe von 100 mg/kg Theophyllin fanden sich bei allen Tieren schwere Herzmuskelnekrosen, [illegible] gleichen Dosis von Coffein waren bei 2 von 10 Tieren leichtere Myocardnekrosen nachweisbar.

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Summary

On the Question of Hepatotoxicity of Caffeine and Theophylline

1. 2 and 16 h after administration of caffeine and theophylline (60 and 100 mg/kg i.p.) to rats the concentrations of free fatty acids, esterified fatty acids, total cholesterine, SGOT and SGPT in the serum, the fat content of the liver and its histological picture were examined.

Both methylxanthines increased the free fatty acids in the serum to the double or triple amount. Following the dose of 60 mg/kg, this effect was observed only after 2 h, following the dose of 100 mg/kg it was still present after 16 h. The concentrations of the esterified fatty acids and of the cholesterine in the serum were not appreciable influenced by caffeine or theophylline.

2 h after administration of 60 mg/kg caffeine and theophylline, SGOT and SGPT were slightly increased. This rise of enzymes, however, proved to be neither dose-dependent nor reproducible.

Only after 100 mg/kg caffeine and theophylline, the lipid content of the liver was found increased by 24% after 16 h. In these animals also fatty degeneration was observed histologically. None of the animals treated with caffeine or theophylline displayed histological evidence of liver cell damage.

2. 24 h after administration of 0.1 ml/kg carbon tetrachloride i.p., SGOT, SGPT, and lipid content of the liver were increased, the concentrations of esterified fatty acids in the serum, however, decreased. Histological inspection revealed liver cell damage as is characteristic for intoxication by CCl_4. The additional administration of caffeine and theophylline (60 mg/kg i.m.) did not influence the changes induced by CCl_4.

3. Since there was no convincing evidence of hepatotoxic effects of methylxanthines, the rise in lipid content of the liver induced by large doses of caffeine or theophylline does not represent liver damage but has to be considered a consequence of lipolysis surpassing the capacity of the liver to metabolise lipids.

4. 16 h after administration of 100 mg/kg theophylline, all the animals showed severe necroses of the cardiac muscle. Following the same dose of caffeine, slighter necroses of the myocardium were observed in 2 out of 10 animals.

Literatur

Atuk, N. O., Blaydes, M. C., Westervelt, F. B. u. Wood, J. F., Circulation 33, 745 (1966)
Bollet, S., Kershbaum, A. u. Aspe, J., Arch. Intern. Med. 116, 750 (1965)
Bollet, S., Kershbaum, A. u. Finck, P. M., Metabolism 17, 702 (1968a)
Bollet, S., Feinberg, L. J., Sandberg, H. u. Hirohayashi, M., J. Pharmacol. exp. Ther. [illegible] (1968b)
Bruns, F. H. u. Wollenweber, J., Klin. Wochr. [illegible]
Butcher, R. W. u. Sutherland, E. W., J. biol. Chem. 237, 1244 (1962)
De Schaepdryver, A. F., Arch. int. Pharmacodyn. 119, 517 (1959)
Dole, V. P., Fat as a tissue, Ed. K. Rodahl, McGraw-Hill, New York, 1964, S. 230
Duncombe, W. G., Clin. chim. Acta 9, 122 (1964)
Eeller, C.-J. u. Ammon, H. P. T., Experientia (Basel) 22, 569 (1966)
Fried, R. u. Hoeflmayr, J., Klin. Wschr. 41, 727 (1963)
Hynie, S., Krishna, G. u. Brodie, B. B., J. Pharmacol. exp. Ther. 153, 90 (1966)
Levi, L., Acta med. scand. 181, 431 (1967)
Reckenagel, R. O., Pharmacol. Rev. 19, 145 (1967)
Schwetz, B. A. u. Plaa, G. L., Toxicol. appl. Pharmacol. 14, 495 (1969)
Steinberg, D., Pharmacol. Rev. 18, 217 (1966)
Stock, K. u. Westermann, E., Naunyn-Schmiedeberg's Arch. Pharmak. exp. Path. 256, 334 (1966)
Strubelt, O., Tillig, K. u. Wolf, F., Med. exp. (Basel) 9, 341 (1963)
Strubelt, O., Breining, H. u. Wüst, H., Arch. Toxikol. 22, 256 (1967)
Trinor, L. u. Nahas, G. G., J. Pharmacol. exp. Ther. 153, 569 (1966)
Ugazio, G. u. Lombardi, B., Lab. Invest. 14, 711 (1965)
Van de Kamer, J. H., ten Bokkel, H. u. Weijers, H. A., J. biol. Chem. 177, 347 (1949)
Watson, D., Clin. chim. Acta 5, 637 (1966)
Zeller, W. u. Ammon, H. P. T., Z. Gastroenterol. 5, 84 (1967)

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CONTINUOUS EXPOSURE OF HeLa CELLS TO CAFFEINE*

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SUMMARY

2 techniques have been used with HeLa cells *in vitro* to determine whether there are cumulative effects of continuous exposure to caffeine at levels ranging from the maximum found in human blood after ingestion of 8 cups of coffee to several times higher. 3 experiments with cells growing in suspension in continuous (chemostat) culture for 7–11 days have shown no increase in incidence of chromatid breaks in the presence of caffeine at 5 and 20 μg/ml. In a second series of experiments, adherent cultures were continuously exposed to caffeine at 3 levels (5, 10 and 20 μg/ml) for 6 and 9 weeks (29 and 48 cell divisions), with subdivision of the cultures and examination of cells for chromatid breaks twice weekly. There were no significant effects on growth, as measured by overall increases in cell numbers, nor in chromatid breaks. Thus, long-term continuous exposure to caffeine at levels several times higher than the transitory peak levels which are normally attainable in man after ingestion of coffee, did not produce any evidence of increased chromatid breaks in 29–48 cell divisions.

INTRODUCTION

Virtually all studies of the cytogenetic effects of caffeine on cells in culture (whether microbial or tissue-derived) have been conducted at concentrations far above the physiological levels attained in the human blood stream after the ingestion of coffee or other caffeinated beverages. For example, in studies on bacterial mutations[8] and inhibition of repair of UV-induced damage to DNA in mammalian cells[10], caffeine concentrations were 50 μg/ml or greater. OSTERTAG, studying chromatid breaks induced in HeLa cells and human leucocytes in culture, did not use concentrations below 500 μg/ml (ref. 9). The *in vitro* studies of KAO AND PUCK[5] with Chinese hamster cells used 16 h exposure to caffeine at levels between 1500 and 8000 μg/ml. In that study, chromatid breaks and rearrangements were observed, but not point

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Abbreviation: MDB, milk dilution bottle.

mutations such as were produced by other agents. (Some specificity may be possible in a specific gene test.)

On the other hand, levels of caffeine in human blood reportedly do not exceed a few μg/ml. The report of AXELROD AND REICHENTHAL[1] showed a maximum of 4.8 μg/ml within 2 h after ingestion of 8 cups of coffee during a 10-h period. Despite the lack of data at levels observed in man, attempts have been made to extrapolate human hazards from the chromosomal effects of caffeine at high levels *in vitro*. The statement has been made that there might be cumulative effects from prolonged exposure to low levels of caffeine[6]. This would appear to be in contradiction to the observation made by the same author that the incidence of chromosome breaks in HeLa cells exposed to high levels of caffeine dropped sharply after 24 h incubation subsequent to the exposure[8].

The present studies were initiated to determine if continuous exposure to levels of caffeine in the range of 5–20 μg/ml would result in the accumulation of chromosomal aberrations. One approach used was the continuous flow culture (chemostat) technique for prolonged cultivation of HeLa cells in suspension in the "steady state"[3]. Further studies were carried out by the propagation and subculture of adherent HeLa cells in the continuous presence of caffeine. Since this study was intended to complement previous data on chromatid breaks induced by caffeine at levels above 500 μg/ml (ref. 9), attention in our study also was directed principally to chromatid breaks and reunion configurations. Moreover, the HeLa cell was used, despite its karyotypic variability, for comparability with the studies of OSTERTAG.

MATERIALS AND METHODS

The HeLa cells used for our studies were supplied by Dr. SHELDON PENMAN of the Department of Biology at Massachusetts Institute of Technology. These cells grow either in suspension culture in spinner flasks or as adherent cultures in bottles and can be passed from one culture mode to the other. Cells were grown in EAGLE's medium[7] and in JOKLIK's modified MEM (Grand Island Biological), with 10% calf serum, penicillin and streptomycin added. Adherent cells are harvested from the bottles with 0.1% trypsin. These cells, when grown adherent in milk dilution bottles (MDB's) have shown a mean doubling time of 33 ± 9 h ($N = 34$). HeLa cells grow equally well in either medium but we found that the chromosomes of mitotic cells did not spread as well after growth in JOKLIK's MEM as in EAGLE's medium. Chromatid breakage data for Table I are derived from JOKLIK's MEM-grown cells and data for Tables II–V from cells grown in EAGLE's medium. Preliminary acute exposure tests with caffeine at levels comparable to those used by OSTERTAG (500–4000 μg/ml) gave comparable incidences of chromatid breaks (0.02–0.03 per chromosome). This cell population appears to have a modal chromosome number of 69, based on counts of 44 cells, values ranging from 62–72. However, because of the uncertainty of this value results are expressed as chromatid breaks per mitosis examined.

Stock solutions of caffeine (Mann Research Laboratories, m.p. 239°) in normal saline were sterilized by Millipore filtration and appropriate amounts added to culture vessels as needed. Our experiments were done with final caffeine concentrations of either 5, 10, or 20 μg/ml. The growth-inhibitory effect of caffeine on HeLa cells was examined in a standard assay[7] over a 3-day period as measured by net protein in-

crease and by cell count. The 50% inhibitory dose by net protein was in the range of 110–140 μg/ml and by cell count was between 135 and 160 μg/ml. These levels are well above those we have used for our studies.

Preparation of samples for microscopic examination of mitotic chromosomes was done essentially by the method of ROTHFELS AND SIMINOVITCH[18], without using colchicine or derivatives. Aliquots of a culture were diluted 1:1 with water, immediately after removal from the caffeine-containing (or control) flask. After 15 min an equal volume of Carnoy's fixative was mixed with the diluted cell suspension. The suspension was centrifuged and the cell pellet washed 2 times with fresh Carnoy's fixative. Clean chilled microscope slides were spotted with a drop of the final cell suspension, and were briefly heated over a Bunsen burner. A drop of aceto-orcein was added and a cover slip placed between the pages of a book of bibulous paper and gentle pressure applied to disrupt the cells and spread the chromosomes. The slides were then examined with a Wild M-20 microscope. At the 1000× magnification used, it is possible to see deletions in chromatids as well as more obvious abnormalities such as reunion configurations. All samples were received by the cytologist under letter-coded designations; they were all observed and quantitated by a single investigator.

Continuous flow cultures of HeLa cells were maintained in water-jacketed 500-ml Bellco spinner flasks using a culture volume of approx. 200 ml. This system was maintained in steady state by controlling the flow rate with a Sigmamotor peristaltic pump. To remove samples for cell counts and chromosome analysis without opening the system, a "built-in" Cornwall syringe was used, taking samples from a point approx. 1 inch from the bottom of the vessel. The culture head space was continuously flushed with a gas mixture of 5% CO_2–95% air, and the culture kept in suspension by means of a magnetic stirrer operating at approx. 200 rev./min. Cell divisions were calculated for each sampling period from the following formula derived from the steady-state growth equation:

$$N_d = (T \times F)/(2.310 \times \ln 2 \times V)$$

where N_d = number of doublings, T = elapsed time in hours, F = flow rate in ml/h, and V = culture volume.

A continuous flow culture was started by inoculating the vessel (at 10^5 cells/ml) with cells that had been grown either in suspension or monolayer. The flow of nutrient was started when the cell density reached $3–5 \cdot 10^5$ cells/ml. The flow rate was adjusted to allow an approximate doubling time of 20–24 h. With a culture volume of approx. 200 ml, this flow rate was 6–8 ml/h.

When cell density reached a constant level, usually after 4–5 days, the chronic exposure to caffeine was initiated by adding a suitable amount of caffeine to the culture vessel and to the nutrient feed stock bottle simultaneously.

The adherent cultures of HeLa cells were grown in small bottles with a culture surface area of approx. 42 square cm to investigate the effects of chronic exposure to caffeine. They were inoculated with $5 \cdot 10^5$ cells per bottle in 25 ml of nutrient. Twice weekly, the cells in each culture were removed by trypsinization, a cell count made to determine growth, a sample prepared for chromosome study, and a fresh subculture started at $5 \cdot 10^5$ cells per bottle with the same concentration of caffeine.

TABLE I

EFFECT OF CAFFEINE ON GROWTH AND CHROMATID BREAKAGE OF HeLa CELLS IN CONTINUOUS CULTURE (Chemostat Expt. 1)

Duration of exposure (days)	Control			Caffeine (5 μg/ml)		
	Cell count per ml (× 10⁵)	Cell divisions (cum.)	Chromatid breaks[a]	Cell count per ml (× 10⁵)	Cell divisions (cum.)	Chromatid breaks
0	6.3		0	5.0		
1	6.2	0.9	0.04	4.6	1.5	0
2	5.2	3.0	0	4.9	2.8	b
3	5.2	4.7	0	5.2	4.3	0.04
4	5.0	6.2	0.04	5.3	5.4	0
5	5.0	7.6	0	6.2	6.3	0
6	5.0	9.2	0.08	5.0	7.3	0.08
7	↓ 4.9[c]	10.5	0	5.0	8.2	0
8	↑ 6.2	10.8	0	4.8	9.4	0.04
9	5.2	12.1	0	4.7	10.4	0
10				4.8	12.0	b
11				4.6	12.8	0.04

[a] Breaks calculated per cell; 25 mitoses examined daily.
[b] Sample lost.
[c] ↑, flow on; ↓, flow off.

RESULTS

Continuous flow

3 separate continuous flow experiments were run with HeLa cells in suspension. These experiments lasted 11, 7 and 7 days, respectively. Cell division in the continuous cultures was very little affected by the presence of caffeine at levels of either 5 or 20 μg/ml, with a suggestion in Expts. 2 and 3 of even a slight acceleration of growth.

Daily examination of 25–30 mitotic cells from each culture in these experiments gave no evidence of a caffeine-induced increase in chromatid breaks. Random

TABLE II

EFFECT OF CAFFEINE ON GROWTH AND CHROMATID BREAKAGE OF HeLa CELLS IN CONTINUOUS CULTURE (Chemostat Expt. 2)

Duration of exposure (days)	Control			Concentration of caffeine					
				5 μg/ml			20 μg/ml		
	Cell count per ml (× 10⁵)	Cell divisions (cum.)	Chromatid breaks[a]	Cell count per ml (× 10⁵)	Cell divisions (cum.)	Chromatid breaks	Cell count per ml (× 10⁵)	Cell divisions (cum.)	Chromatid breaks
0	3.6		0	3.6		0	3.2		0.06
1	3.6	0.9	0	2.9	1.5	0	2.7	0.9	0
2	↓ 3.6[c]	2.0	b	↓ 2.6	3.0	b	↓ 2.6	2.0	b
3	3.4	2.8	0	2.6	4.0	0	2.4	2.5	0.03
4	↑ 5.9	3.6	0.03	↑ 5.0	4.9	0.03	↑ 5.0	3.6	0.06
5	5.0	4.4	0	4.5	5.8	0.03	4.6	4.4	0.03
6	5.0	5.2	0.06	4.6	6.0	0.06	4.6	5.8	0.03
7	4.8	6.0	0	4.5	7.8	0.06	4.4	6.8	0.06

[a] Breaks calculated per cell; 30 mitoses examined daily.
[b] Sample not taken.
[c] ↑, flow on; ↓, flow off.

TABLE III

EFFECT OF CAFFEINE ON GROWTH AND CHROMATID BREAKAGE OF HeLa CELLS IN CONTINUOUS CULTURE (Chemostat Expt. 3)

Duration of exposure (days)	*Control*			*Concentration of caffeine*					
				5 µg/ml			*20 µg/ml*		
	Cell count per ml (× 10⁵)	*Cell divisions (cum.)*	*Chromatid breaks*[a]	*Cell count per ml (× 10⁵)*	*Cell divisions (cum.)*	*Chromatid breaks*	*Cell count per ml (× 10⁵)*	*Cell divisions (cum.)*	*Chromatid breaks*
0	4.6		0	4.7		0	4.5		0
1	4.8	0.8	0	4.4	1.2	0	4.4	1.2	0.069[b]
2	4.6	1.7	0	4.3	2.3	0.067	4.4	2.6	0.067
3	4.0	2.6	0	↓ 1.8[d]	2.7	0	↓ 1.0[d]	3.0	0
4	4.4	3.4	0	2.2	3.9	0.10[c]	2.3	4.2	0
5	4.6	4.3	0	↑ 4.0	4.8	0	↑ 4.2	5.1	0
6	4.2	5.4	0	3.8	5.7	0	4.0	6.3	0
7	↓ 2.8[e]	6.0		↓ 1.0	6.5		↓ 1.0	6.9	

[a] Breaks calculated per cell: 30 mitoses examined except where indicated.
[b] 29 mitoses.
[c] 10 mitoses.
[d] Stirrer off overnight, turned back on.
[e] ↑, flow on; ↓, flow off.

distribution of 1 or 2 breaks per 25 or 30 mitoses was observed throughout control and experimental groups (Tables I–III). To facilitate comparison of experiments, these results are expressed as breaks per mitosis (or cell). The chromatid aberrations observed were exclusively chromatid breaks. There were no reunion figures observed.

Because of the low incidence of breaks, statistical analysis of these data for significance has required the transformation of the data on the assumption of a Poisson distribution[13]. After transformation, pair-wise comparison of each treated group with a proper control gave nonsignificant values of the F ratio.

TABLE IV

EFFECT OF CAFFEINE ON GROWTH AND CHROMATID BREAKAGE OF HeLa CELLS IN CONTINUOUS CULTURE (Continuous culture for 9 weeks, subcultured twice weekly.

Weeks	*Control*		*Concentration of caffeine*					
			5 µg/ml		*10 µg/ml*		*20 µg/ml*	
	Cum. divisions	*Chromatid breaks*	*Cum. divisions*	*Chromatid breaks*	*Cum. divisions*	*Chromatid breaks*	*Cum. divisions*	*Chromatid breaks*
1	5.2	—	4.6	—	5.2	—	4.8	—
2	9.7	—	9.0	—	9.7	—	7.4	—
3	13.4	—	12.1	—	12.5	—	10.4	—
4	18.9	1, 0	16.4	1, 0	16.4	1, 1	15.4	2, 1
5	24.9	2, 1	22.2	1, 0	22.3	2, 2	21.6	2, 2
6	31.0	0, 2	28.4	0, 0	28.5	0, 1	24.9	1, 0
7	36.6	0, 2	33.1	2, 2	34.2	1, 0	28.4	0, 0
8	41.7	2, 0	38.9	0, 1	40.2	1, 1	32.6	0, 0
9	47.8	0, 0	45.7	0, 2	46.5	0, 0	39.3	1, 0
Total breaks		10		9		10		9
Breaks/cell[a]		0.028		0.025		0.028		0.025

30 mitoses examined per sample, 2 samples per week.
[a] 30 mitoses × 12 observations = 360 mitoses examined per caffeine level.
—, cells not spreadable; changed to EAGLE's medium after 3 weeks.

Adherent cultures

2 experiments were conducted with glass-adherent HeLa cells. 1 of these experiments had a duration of 9 weeks (Table IV) and the second, with cultures in sextuplicate at each caffeine level, of 6 weeks (Table V). The effect of caffeine on growth of the cells at the 5 and 10 μg/ml levels was insignificant, although a lower number of divisions was observed in both experiments at 20 μg/ml. Analysis by *t*-test of the growth data at 6 weeks in the second experiment shows that the difference between the controls and the 20 μg/ml cultures is not significant. This analysis was not possible in the first experiment because of insufficient culture replication.

TABLE V

EFFECT OF CAFFEINE ON GROWTH AND CHROMATID BREAKAGE OF HeLa CELLS IN CONTINUOUS CULTURE

6 replicate cultures at each dose level, including control, exposed to caffeine continuously for 6 weeks, analysed and subcultured twice weekly.

Weeks	*Control*			*Concentration of caffeine*								
				5 μg/ml			*10 μg/ml*			*20 μg/ml*		
	Cum. divisions	*N*[b]	*Chromatid breaks*[a]	*Cum. divisions*	*N*	*Chromatid breaks*	*Cum. divisions*	*N*	*Chromatid breaks*	*Cum. divisions*	*N*	*Chromatid breaks*
1	3.9 ± 0.6	12	0.011	3.9 ± 0.5	11	0.027	4.2 ± 0.9	11	0.015	3.1 ± 0.8	11	0.009
2	8.6 ± 1.6	11	0.015	9.0 ± 0.8	12	0.019	9.3 ± 1.5	12	0.011	8.2 ± 1.6	12	0.014
3	14.0 ± 2.0	12	0.006	14.7 ± 1.3	12	0.011	12.5 ± 5.4	12	0.019	12.9 ± 1.2	12	0.019
4	19.0 ± 4.7	10	0.020	19.0 ± 3.2	10	0.013	19.7 ± 4.6	11	0.027	17.6 ± 4.0	11	0.018
5	24.8 ± 5.5	12	0.019	25.6 ± 4.9	12	0.017	25.6 ± 5.2	12	0.033	22.5 ± 6.0	12	0.022
6	29.0 ± 6.0	8	0.025	29.4 ± 5.5	8	0.037	29.6 ± 5.8	8	0.008	26.5 ± 6.7	8	0.008
Weekly mean ± S.D.			0.016 ± 0.0068			0.021 ± 0.0098			0.019 ± 0.0096			0.015 ± 0.0057

Divisions calculated twice weekly as $D = 3.32 * \log (C_2/C_1)$, where C_1 = inoculum, C_2 = cell count at subculturing

[a] Breaks per cell.

[b] *N*, number of samples examined. Therefore, number of cells examined per week per dose level = *N* × (30 mitoses per sample).

The incidence of chromatid breaks in these cultures as observed twice weekly for each dose level, appeared to vary randomly with no evidence of a consistent trend with either time or dose level. In the second experiment (Table V), individual weekly averages were compared statistically to the corresponding controls, again with the data appropriately transformed. No significant *F* values were found. The chromatid breaks seen in these cells were like those seen in the chemostat experiments with no difference from those seen in control cells.

DISCUSSION

With due regard for the influence that physiological factors such as cell uptake, metabolism and distribution may have in predicting *in vivo* effects from *in vitro* studies, the present study was based on the premise that if estimations of human hazard are to be made from *in vitro* data, the levels of exposure *in vitro* should bear a reasonable relationship to levels attainable *in vivo*. Furthermore, despite the usual caveat that effects at low levels should be predictable from high-level positive effects,

it was a deliberate attempt to look for cumulative effects of continuous exposure. The levels of caffeine selected for continuous exposure were 5 μg/ml, the maximum blood level observed in human subjects under normal conditions of ingestion of caffeine-containing beverages, and 10 and 20 μg/ml. The maximum blood level is, of course, only transient, with a caffeine half-life time of about 3.5 h (ref. 1). The figure cited (from AXELROD AND REICHENTHAL) was based on caffeine blood levels in 5 subjects drinking 2 cups of coffee 4 times during a day (at h 0, 4, 7 and 10) with blood samples taken at h 0, 1, 5, 11 and 24. Calculation of the area under the average blood level curve for the 24-h period yields a daily exposure value ($C \times t$) for cells in equilibrium with the body fluids of 50–60 μg-h/ml caffeine. The data of AXELROD AND REICHENTHAL and others indicate that caffeine is distributed to all tissues with body water. Although the levels used were greater than the maximum transient *in vivo* level by a factor of not more than 4, they yielded daily $C \times t$ values of 120, 240 and 480 μg-h/ml. The highest value was 8–10 times greater than the *in vivo* $C \times t$ value, and was equivalent to that produced by 1-h exposure to caffeine at 500 μg/ml, which *in vitro* gave a 7-fold increase in chromatid breaks per chromosome[9].

Cultures were exposed at these rates for up to 48 cell generations without major effects on growth and without increasing the incidence of chromatid breaks. This is in marked contrast to OSTERTAG's experience, also with HeLa cells, using high levels (500 μg/ml and higher) of caffeine for periods of 1 or 2 h, and suggests that there is a minimum effective concentration (above that obtainable in man) required to produce chromatid breaks. It has been estimated that our techniques and analysis are adequate to detect a 2-fold increase in chromatid breaks. The fact that such an increase was not found is consistent with the *in vivo* observations of ROHRBORN[11] and EPSTEIN *et al.*[4], that acute or chronic administration of caffeine did not produce an increase of dominant lethal mutations in mice.

REFERENCES

1 AXELROD, J., AND T. REICHENTHAL, The fate of caffeine in man and a method for its estimation in biological material, *J. Pharmacol. Exptl. Therap.*, 107 (1953) 519.
2 CLEAVER, J. E., Repair replication of mammalian cell DNA; Effects of compounds that inhibit DNA synthesis or dark repair, *Radiation Res.*, 37 (1969) 334–348.
3 COHEN, E. P., AND H. EAGLE, A simplified chemostat for the growth of mammalian cells; Characteristics of cell growth in continuous culture, *J. Exptl. Med.*, 113 (1961) 467–474.
4 EPSTEIN, S. S., W. BASS, E. ARNOLD AND Y. BISHOP, The failure of caffeine to induce mutagenic effects or to synergize the effects of known mutagens in mice, *Food Cosmet. Toxicol.*, 8 (1970) 381–401.
5 KAO, F. T., AND T. T. PUCK, Genetics of somatic mammalian cells, IX. Quantitation of mutagenesis by physical and chemical agents, *J. Cell Physiol.*, 74 (1969) 245–257.
6 KUHLMANN, W., H. FROMME, E. HEEGE AND W. OSTERTAG, The mutagenic action of caffeine in higher organisms, *Cancer Res.*, 28 (1968) 2375–2389.
7 LEVINTOW, L., AND H. EAGLE, The technique of mammalian cell culture, in S. P. COLOWICK AND N. O. KAPLAN (Eds.), *Methods in Enzymology*, Vol. 5, Academic, New York, 1962, pp. 77–89.
8 NOVICK, A., Mutagens and antimutagens, *Brookhaven Symp. Biol.*, 8 (1955) 201–215.
9 OSTERTAG, W., E. DUISBERG AND M. STÜRMANN, The mutagenic activity of caffeine in man, *Mutation Res.*, 2 (1965) 293–296.
10 RAUTH, A. M., Evidence for dark-reactivation of ultraviolet light damage in mouse L cells, *Radiation Res.*, 31 (1967) 121–138.
11 ROHRBORN, G., Mutagenicity tests in mice, *Humangenetik*, 6 (1968) 345–361.
12 ROTHFELS, K. H., AND L. SIMINOVITCH, An air-drying technique for flattening chromosomes in mammalian cells grown *in vitro*, *Stain Technol.*, 33 (1968) 73–77.
13 STEEL, R. G. D., AND TORRIE, J. H., *Principles and Procedures of Statistics*, McGraw Hill, 1960.

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Genetic Tests in Mice of Caffeine Alone and in Combination with Mutagens

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Genetic Tests in Mice of Caffeine Alone and in Combination with Mutagens. THAYER, P. S. AND KENSLER, C. J. (1973). *Toxicol. Appl. Pharmacol.* 25, 157–168. The possible mutagenicity of caffeine has been studied in mice by the dominant-lethal method, in three experiments. (I) Male mice were given caffeine in drinking water for 8 weeks at 3.6, 13.4, 49, and 122 mg/kg/day (comparable to human consumption of 2.8–95 cups of coffee per day). Subsequent mating of each of six males from each group to five females per week for 8 weeks showed no significant increase in dominant-lethal mutations (embryonic deaths) whether expressed as early deaths per pregnant female or as mutation index. Although males consuming the two higher levels of caffeine produced fewer pregnancies, litter sizes of females giving birth were not reduced. (II) Single ip injections of caffeine (15 mg/kg) were given to groups of male mice prior to, subsequent to, and immediately at the time of receiving X-rays (100 r). Each of five males from each group was mated to five females per week for 7 weeks. Embryonic deaths did not show any enhancing effect of caffeine on the mutagenicity produced by the irradiation. (III) Three groups of male mice ingested caffeine in water for 16 weeks at levels of 0, 4 and 13 mg/kg/day. Subgroups of five from each group were given either: no further treatment, a single dose of triethylene melamine at 0.2 mg/kg, or 100 r of X-ray, and mated for 7 weeks as above. Fertility and litter size were not affected by the caffeine pretreatment, nor did it modify the induction of dominant-lethal mutations by triethylene melamine or X-rays. Litter sizes showed no significant preimplantation losses in any experiment. Thus, under the conditions described herein and at the doses employed (higher than human exposure), there was no evidence for the mutagenicity of caffeine or the inhibition of DNA repair mechanisms in these mammalian systems.

Recent concern with the possible genetic effects of caffeine in man has stimulated a number of studies in mammalian systems felt to be more directly applicable to man than studies in bacterial, plant and cell culture systems. The earlier studies and the mammalian results have been reviewed in connection with the publication of negative mammalian data by Röhrborn (1968), Epstein *et al.* (1970) and Adler (1970). Briefly, the mutagenic studies of caffeine can be summarized as follows:

a. Mutagenic effects have been observed in certain bacterial systems.

b. In vitro studies involving plant, animal or human cells have shown that high levels of caffeine can cause chromosome damage.

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c. Several carefully performed experiments have shown neither direct effects in mammalian systems as measured by the dominant-lethal procedure of Bateman (1966), nor enhancement of rates of dominant-lethal mutations induced by known mutagens (Epstein *et al.*, 1970). Reservations have been expressed (Adler, 1970) about the apparently positive results of Kuhlmann *et al.* (1968).

The present studies, using the dominant-lethal method, were designed to test the direct effect of caffeine after continuous administration in drinking water and its possible indirect enhancing effect on the induction of mutations by X-radiation (from ^{60}Co) and by a chemical mutagen (triethylene melamine). These studies included levels of caffeine intake from 3.6 to 122 mg/kg/day. Persons drinking three to six cups of coffee a day and weighing approximately 70 kg would have an average caffeine intake of 3.8–7.7 mg/kg/day.

METHODS

Animals. All mice used were of the HA/ICR strain, obtained from Charles River Laboratories, as CD-1 mice, or bred in our own laboratories by random breeding from such purchased stock. Because of the large numbers of females required it was not feasible to use animals of proved fertility.

Treatment. Solutions of caffeine (Mann Research Laboratories, mp 239°C) were made up weekly in distilled water for ingestion experiments and as needed for injection. Triethylene melamine (TEM) was obtained from the National Cancer Institute.

When caffeine was administered in drinking water, measurement of fluid intake was made. This was done for the full duration of the first experiment and at intervals during the enhancement experiment.

Irradiation was performed in a ^{60}Co source at a dose rate of approximately 60 r/min. The dose selected for these experiments, 100 r of whole body radiation, was chosen after a preliminary assessment of the effects of doses in the range of 50–300 r. The radiation level of 100 r was chosen to give a significant increase in dominant lethal mutations but was still moderate enough to permit the detection of enhancement by caffeine, if produced.

Experimental designs. Three separate experiments have been conducted, one to assess the effects of caffeine itself and two on the possible enhancement of mutagenicity of other agents.

In the first experiment, four groups of male mice were given caffeine in drinking water (at 0.02, 0.06, 0.2, and 0.6 mg/ml) for a period of 8 weeks and a fifth group of controls received no caffeine. Following this, six males from each group, without further ingestion of caffeine, were each mated to five females per week for a further 8 weeks.

The second experiment was an irradiation enhancement study. Acute doses of caffeine (15 mg/kg) were administered by single ip injection to separate groups of males as follows: 0.5–1 hr prior to irradiation, immediately (a few minutes) prior to irradiation and 0.5–1hr subsequent to irradiation. Irradiation was administered to randomly selected males from each of these three groups and a control group. In addition, a caffeine control (not irradiated) and an untreated control group were included. Five males from each of the six groups were each mated to five females weekly for 7 weeks.

The third experiment involved the semichronic administration of caffeine in drinking water followed by irradiation or TEM injection. Male mice were given caffeine at 0.02 and 0.06 mg/ml, and a control group received tap water, for a period of 16 weeks. At the end of this time each pretreatment group was divided into subgroups for (1) control purposes, (2) treatment with 100 r X-ray, and (3) treatment with a single ip injection of TEM at 0.2 mg/kg. Five males from each of the nine subgroups were mated to five females per week for a period of 7 weeks.

Observation and analysis. Embryos were observed 12–14 days after the detection of vaginal plugs in the mothers and counts of both apparent early deaths and late deaths were made, following the criteria of Bateman and Epstein (1971). Results of this count are expressed as early deaths/pregnant female (ED/P), following the method of Epstein, and as mutation index (MI) following the method of Bateman. The MI is the sum of the numbers of early deaths and late deaths divided by the total number of implants. We did not count corpora lutea, as being "notoriously difficult, laborious and inaccurate in mice" (Epstein *et al.*, 1970). For assessment of preimplantation losses, we have relied upon calculation of mean implant number per female. This question is also examined by intergroup comparison of distribution of numbers of females by number of total implants. Similarly, assessment of embryonic death data is not based solely on comparison of ED/P or MI values but also on intergroup comparison of distribution of numbers of females by number of dead implants.

RESULTS

Caffeine Effects after 8 Weeks Administration in Drinking Water

At the levels of caffeine administered in our experiment, fluid intake was slightly increased in the caffeine-treated groups as compared to the control groups (Table 1). This is contrary to the experience of Epstein *et al.* (1970). The somewhat lower body weight gain of the animals at the highest dose may be a reflection of their slightly higher initial weight.

TABLE 1

SEMICHRONIC CAFFEINE EXPERIMENT: CAFFEINE INTAKE AND BODY WEIGHTS IN MICE[a]

Dose level	Average consumption[b]				Mean body weight (g)					
	Water (ml/kg/day)		Caffeine (mg/kg/day)		Original		Final		Δ	
(mg/ml)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	162	23			34.9	2.2	43.4	3.4	+8.5	4.5
0.02	178	27	3.6	0.55	35.0	3.4	39.7	4.9	+4.7	5.6
0.06	206	23	12.4	1.4	37.2	2.3	42.4	4.3	+5.2	4.2
0.2	246	48	49.2	9.6	36.9	2.2	42.8	2.6	+5.9	3.3
0.6	203	32	121.6	19	38.0	2.5	42.1	3.0	+4.1	4.3

[a] Fifteen male CD-1 mice/group were given caffeine in drinking water for 8 weeks. Liquid consumption was determined weekly and averaged to daily rate.

[b] Based on 8 weekly measurements.

Pregnancy rates and average total implants are shown in Table 2. The only significant effect of caffeine was the apparent lower fertility of males at the two higher caffeine levels (49 and 122 mg/kg/day). This effect has been noted previously by other authors (Epstein *et al.*, 1970). However, the females in these two groups which were successfully impregnated had total implant numbers similar to those for lower doses and controls. It would appear, then, that these very high levels of caffeine (equivalent to the caffeine in 40 and 100 cups of coffee per day) influence male mating behavior. The grouping of females by number of total implants was also consistent with an all-or-none effect on fertility. Chi-square analysis comparing each of the distributions for the caffeine-treated groups with the control group showed no significance, with p values of 0.1 or larger for all groups. In each group, except at the highest caffeine level, there were a few females with small numbers of implants, i.e., 5 or less. This is more likely a reflection of the fact that these were primiparous females rather than an effect of caffeine on the male. The differences were not dose-related and there was one female within the control group with five implants.

TABLE 2

SEMICHRONIC CAFFEINE EXPERIMENT: PREGNANCY AND IMPLANT VALUES IN MICE[a]

	Pregnancies (%)[b]			Implants per Female		
		Overall			Overall	
	Weekly range	Mean	SD	Weekly range	Mean	SD
Control	33–67	50	12	9.7–12.1	10.9	0.9
0.02 mg/ml	27–63	45	12	9.1–12.8	10.4	1.1
0.06 mg/ml	17–70	46	18	9.2–11.9	10.4	1.0
0.2 mg/ml	17–50	29	11	9.6–12.3	10.5	0.9
0.6 mg/ml	10–37	23	8	10.0–12.8	11.0	1.1

[a] Six males from each group mated with five females per week for 8 weeks after 8 weeks ingestion of caffeine (see Table 1).
[b] Percentage based on 30 matings per week, 240 in 8 weeks.

Data for embryonic deaths, expressed both as ED/P and MI, are presented in Table 3. Overall ED/P and MI values for the whole mating period show no significant effects produced by any level of caffeine. The two highest levels even appear to have given a decrease in embryonic deaths by either of these parameters, although the total implants involved were lower. Inspection of the weekly data shows a sporadic incidence of high values for each parameter, namely for controls in the fifth week, 0.02 mg/ml in the seventh week and 0.06 mg/ml in the eighth week. Each of the latter two is largely attributable to a single female with a large number of embryonic deaths. Such clustering may be due to factors other than mutation during spermatogenesis (Röhrborn, 1968). There was no trend toward a weekly peak of embryonic deaths at any dose level, the numbers varying randomly within or near the range shown by the control group. Although the week-by-week numbers of dead implants are relatively small, the number of total implants would be sufficient to show effects of an active mutagen as was found with irradiation and TEM (see next sections).

TABLE 3

SEMICHRONIC CAFFEINE EXPERIMENT: EMBRYONIC DEATHS IN MICE

	Caffeine (mg/ml)														
	0			0.02			0.06			0.2			0.6		
Week	ED	ED/P	MI[a]	ED	ED/P	MI	ED	ED/P	MI	ED	ED/P	MI	ED	ED/P	MI
1	3[e]	0.15	1.3	3	0.23	2.2	7	0.43	4.6	3	0.20	2.0	3	0.43	4.2
2	4	0.25	2.5	10	0.53	5.1	4	0.27	2.7	0	0.0	0.0	5	0.45	4.4
3	4	0.31	2.8	4	0.24	2.4	8[e]	0.38	3.7	1	0.10	1.0	1	0.20	1.9
4	4	0.31	3.2	0	0.0	0.0	1	0.20	2.1	3	0.50	4.8	0	0.00	0.0
5	9[b]	0.90	9.0	9	0.60	6.0	7	0.37	4.0	1	0.20	2.1	0	0.00	0.0
6	5	0.42	3.8	3	0.21	2.1	5	0.71	6.6	3	0.30	2.8	1	0.14	1.3
7	8	0.40	3.5	15[c]	1.15	10.0	7	0.47	3.9	2	0.18	1.6	4	0.50	4.1
8	10	0.62	5.2	7	0.78	6.1	14[d,e]	1.08	9.1	1	0.17	1.4	3	0.33	2.6
Overall	47	0.39	3.6	51	0.47	4.5	53	0.48	4.6	14	0.20	1.9	17	0.31	2.8

[a] ED = early deaths, LD = late deaths, P = number of pregnant females, MI = (ED + LD)/Imp × 100. See Table 2 for numbers of pregnant females and total implants.

ED/P values for individual females.

[b] 3/14, 2/9, 2/6, 1/9, 1/10; 5 females with none dead.

[c] 8/13, 2/12, 2/11, 1/12, 1/11, 1/8; 7 females with none dead.

[d] 5/11, 2/13, 1/16, 1/14, 1/13, 3 × 1/11, 1/10; 4 females with none dead.

[e] Includes one "late death."

The distribution of females by number of dead implants totaled over the whole 8-week period, were compared to values for a Poisson distribution at the same total incidence. The observed and calculated values are close and in a few cases the number of females with single dead implants, e.g., in the 0.02 mg/ml group, is less than predicted and the number with no dead implants is greater. In such a group, the incidence is distorted by the clustering of deaths in aberrant females.

Acute Caffeine Administration on Mutations Induced by Irradiation

In this experiment, irradiation alone appeared to give a slight reduction in fertility and in mean implant number (Table 4). This effect was not increased by caffeine. Caffeine alone gave values comparable to the controls. Week-by-week data do not show any significant differences in implant number between experimental groups, using the X-ray group as a control for the X-ray plus caffeine groups.

TABLE 4

COMBINATION X-RAY (^{60}Co) PLUS ACUTE ADMINISTRATION OF CAFFEINE: PREGNANCY AND IMPLANTATION IN MICE

	Pregnancies (%)[a]			Implants per female		
	Weekly range	Overall Mean	Overall SD	Weekly range	Overall Mean	Overall SD
Control	36–68	47	10	11.1–12.5	11.9	0.5
X-ray[b]	20–60	34	15	9.4–11.4	10.2	1.0
Caffeine[c]	32–52	43	8	10.1–12.3	11.2	0.8
X-ray + caffeine[d]						
$t_0 - 1$ hr	24–68	43	15	9.5–11.3	10.5	0.6
t_0	32–48	39	6	9.4–12.7	11.1	1.1
$t_0 + 1$ hr	28–56	43	9	9.2–11.5	10.5	0.7

[a] Five males in each group mated to five females per week. Percentage is based on 25 matings per week, 175 in 7 weeks.
[b] 100 r from ^{60}Co source.
[c] 15 mg/kg, single ip injection.
[d] Caffeine injected at time indicated relative to irradiation.

The data on embryonic deaths, presented in Table 5, show that irradiation increased early deaths, but that caffeine had no effect. The values for all weeks combined indicate that caffeine alone did not give a significant increase by either criterion. Caffeine treatment preceding, at the time of, or following irradiation did not produce any change in embryonic death rates over that observed in the group receiving irradiation only. For each of the four groups which received irradiation there was a moderately high incidence of dead embryos during the first week, dropping in the second week, followed by even higher values in the third, fourth and fifth weeks with a marked decrease in the sixth and seventh weeks. There was variation in the time when the peak value appears. This may be largely attributed to minor fluctuations in time between mating and sacrifice which are inherent in such an experiment.

TABLE 5

COMBINATION X-RAY (^{60}Co) PLUS ACUTE ADMINISTRATION OF CAFFEINE: EMBRYONIC DEATHS IN MICE

										X-ray + caffeine[a]								
	None			X-ray[a]			Caffeine[a]			$t_0 - 1$ hr			t_0			$t_0 + 1$ hr		
Week	ED	ED/P	MI[c]	ED	ED/P	MI	ED	ED/P	MI	ED	ED/P	MI	ED	ED/P	MI	ED	ED/P	MI
1	4	0.44	3.9	4	0.80	7.8	2	0.25	2.4	6	1.00	9.2	10	1.25	10.9	10	0.91	8.7
2	5	0.42	3.4	1	0.20	2.1	6	0.46	4.2	10[b,b]	0.59	6.6	7[b]	0.64	6.9	8[b]	0.73	7.5
3	10	1.00	8.4	7	1.40	14.6	3[b]	0.33	3.8	11	1.57	15.7	17	1.42	15.0	9	1.29	11.5
4	9[b]	0.75	6.9	11[b]	1.22	12.2	2[b]	0.18	2.2	11	1.00	10.0	9	0.90	7.1	14[b]	1.27	11.8
5	9	0.82	6.5	18	2.00	17.5	6[b]	0.46	4.7	19	1.46	12.9	12	1.20	10.0	13	1.44	13.3
6	7	0.41	3.7	7	0.64	7.4	8	0.89	8.8	10	1.00	10.5	5	0.88	5.8	4	0.31	3.3
7	7	0.58	4.9	13	0.87	8.0	9	0.75	6.6	12	1.00	9.2	6[b]	0.67	7.1	6	0.43	4.2
Total	51	0.61	5.3	61	1.03	10.3	36	0.48	4.6	79	1.04	10.1	66	0.97	9.0	64	0.84	8.2

[a] See Table 4.
[b] Plus one "late death."
[c] MI = (ED + LD)/Imp × 100 (see Table 3).

The distributions of numbers of females by number of embryonic deaths was compared with distributions calculated from the Poisson distribution. In several cases, the number of females with no embryonic deaths was somewhat larger than predicted and the number with one dead embryo smaller than expected, while the values for larger numbers of embryonic deaths are close to the predicted values. Other groups showed close agreement with the expected values.

The distributions of total implants in the X-ray plus caffeine groups were comparable to those for both the caffeine and X-ray control groups, and Chi-square tests showed no significant differences between any of the combination groups and either of the positive controls. This observation indicates that preimplantation loss is not a factor in these results.

Semichronic Caffeine Administration plus TEM and X-ray

Prior to treatment with TEM or X-ray, male animals were given caffeine in drinking water at 0, 0.02, and 0.06 mg/ml for a period of 16 weeks. Body weight changes and fluid intake values for the first, fourth and eighth weeks are presented in Table 6. This shows a consistent pattern of weight gain for both treated groups as compared to the controls and insignificant change in fluid intake for any group over the period of measurement. The overall consumption rates for caffeine were about 4 and 13 mg/kg/day.

TABLE 6

MUTAGENESIS AFTER CAFFEINE INGESTION: CAFFEINE INTAKE AND BODY WEIGHT OF MALE MICE[a]

Week	Body wt (g)	Control (ml/day)	Body wt (g)	Caffeine 0.02 mg/ml		Body wt (g)	Caffeine 0.06 mg/ml	
				ml/day	mg/kg/day		ml/day	mg/kg/day
1	35.0	8.6	35.8	6.7	3.8	34.6	7.2	12.5
4	36.6	7.8	37.1	7.7	4.2	36.5	7.6	12.5
8	37.8	8.6	38.0	7.6	4.0	37.2	8.1	13.1

[a] Mean values for groups of 30 males, given caffeine in drinking water for 16 weeks.

Each of the three pretreatment groups was divided into three subgroups of five males each, one of which was treated with 100 r of X-ray, a second with TEM (0.2 mg/kg) and the third retained as a control group. The pregnancy and implant values obtained show no significant effects of the caffeine treatment (Table 7). The caffeine-treated animals gave a somewhat higher fertility rate, particularly in the TEM treatment groups and in the X-ray group which received the higher dose of caffeine. However, since the overall pregnancy rates (percentage of the 175 females mated in each subgroup) were low, ranging from 18 to 42%, this could well be due to chance variation. There are no notable differences in weekly values for mean number of implants with only sporadic variations occurring from group to group.

Caffeine pretreatment did not affect the mutagenic activity of TEM or of X-ray, when these rates are considered either as ED/P or MI (Table 8). This is seen both in the overall totals and week by week. To make allowance for the somewhat variable responses and

TABLE 7

MUTAGENESIS AFTER CAFFEINE INGESTION: PREGNANCY AND IMPLANTATION IN MICE[a]

	Pretreatment group					
	Control		Caffeine (0.02 mg/ml)		affei ne (0.06 mg/ml)	
Mutagen	Pregnancy[b] (%)	Mean total implants	Pregnancy (%)	Mean total implants	Pregnancy (%)	Mean total implants
None Weekly	12–48	9.2–12.4	8–36	9.9–13.5	20–40	10.8–12.2
Overall	26	11.1	23	11.4	29	11.2
TEM Weekly	20–40	10.0–12.2	28–50	9.3–12.0	28–52	8.5–12.6
Overall	28	10.9	35	10.7	39	10.4
X-ray Weekly	4–28	9.0–14.0	16–36	9.8–11.6	28–64	8.8–11.6
Overall	18	11.0	24	10.6	42	10.4

[a] Five males in each group mated to five females per week for 7 weeks.
[b] Pregnancy percentage based on 25 matings per week, 175 in 7 weeks.

TABLE 8

MUTAGENESIS AFTER CAFFEINE INGESTION: EMBRYONIC DEATHS IN MICE

		Control			Caffeine (0.02 mg/ml)			Caffeine (0.06 mg/ml)		
Mutagen	Week	ED	ED/P	MI[b]	ED	ED/P	MI	ED	ED/P	MI
None	1	1	0.20	1.8	5	2.50	18.5	5	1.00	9.3
	2	5	1.00	8.1	1	0.14	1.2	4	0.50	4.6
	3	1	0.33	3.1	0	0.0	0.0	2[c]	0.40	4.9
	4	2[c]	0.17	1.5	5	0.56	4.6	4	0.40	3.6
	5	3	0.38	4.3	8	0.89	9.0	8	1.00	8.7
	6	4	0.57	5.3	2	0.67	6.3	5[c]	0.71	7.7
	7	2	0.40	4.3	2[c]	0.33	4.8	5	0.63	5.7
	Total	18	0.40	3.8	23	0.56	5.2	33	0.65	6.1
TEM	1	14	2.80	24.1	19	2.39	22.1	8	1.00	11.8
	2	21	3.00	30.0	39	4.33	46.4	16	2.29	21.3
	3	31[c]	3.88	33.7	31[c]	3.10	31.4	23	2.09	23.2
	4	6	1.20	9.8	10	1.43	13.5	6[c]	0.55	5.6
	5	1	0.17	1.6	3	0.27	2.3	4	0.50	4.0
	6	6	0.60	5.7	6	0.67	5.7	5	0.45	4.2
	7	4	0.50	4.7	1	0.13	1.3	1	0.08	0.8
	Total	83	1.69	15.7	109	1.76	16.7	63	0.91	8.9
X-RAY	1	5	0.83	6.9	2	0.50	5.0	2	0.29	3.3
	2	3	0.60	5.4	4	0.57	5.1	8	0.89	7.9
	3	14	2.00	16.5	8	1.60	15.1	20	1.82	16.0
	4	0	0.0	0.0	8	1.60	13.8	17	1.70	14.7
	5	6	1.50	14.6	7	1.17	10.9	14	0.88	8.6
	6	2	1.00	11.1	5	0.83	8.5	6[c]	0.50	5.6
	7	1	0.14	1.5	5	0.56	5.3	2	0.25	2.9
	Total	31	0.97	8.8	39	0.93	8.7	69	0.95	9.2

[a] See Table 7 for numbers of pregnant females and total implants, and treatment.
[b] MI = (ED + LD)/Imp × 100 (see Table 3).
[c] Plus one "late death."

the possible errors of timing of the matings relative to the time of treatment, bi-weekly moving averages have been calculated (but are not shown here). This results in smoothing and still shows clearly the lack of effect of the caffeine pretreatment. The animals pretreated with the higher caffeine level show lowered response to TEM, and an increase in fertility. The first week's data for the group receiving 0.02 mg/ml caffeine and no mutagen appear high, but since there were only two females in this group (the lowest number pregnant in any week of this set) the significance of this point is doubtful. The mean values for the first 2 weeks for this group and the control group are not significantly different.

The distribution of females by number of dead implants showed similar values in the three subgroups which received no mutagen with the exception of a few excess females with one dead implant in the 0.06 mg/ml caffeine group. Chi-square tests showed that neither caffeine subgroup differed from the control. In the TEM group, the noncaffeine and the 0.02 mg/ml subgroups had higher numbers of animals with more than one dead implant, a tendency which was not as clear in the 0.06 mg/ml caffeine subgroup. All three TEM-treated subgroups had dead implant distributions significantly different from the Poisson distribution, but not from each other. In the irradiated groups the patterns of distribution were quite similar for control and caffeine-treated subgroups, and neither of the latter was different from the control.

The mean implant data and the distribution of implant data are both clearly indicative of no significant preimplantation losses. In the 0.06 mg/ml caffeine subgroups for both TEM and X-ray treatment there was a scattering of a few animals with five or fewer implants. However, this was more than compensated for, on a statistical basis, by the incidence of females with normal or higher numbers of implants. Furthermore, Chi-square comparison of each caffeine treated subgroup with its respective control showed no significant difference attributable to caffeine pretreatment.

DISCUSSION

Caffeine, administered to male mice for periods of 8–16 weeks at levels approximating or greatly exceeding that ingested in the human consumption of coffee did not show a mutagenic effect. Likewise, under the conditions of the experiment, caffeine did not enhance the mutagenic effects of TEM or X-rays. The similar negative experiments of Epstein (1970), involved larger doses of caffeine with ingestion levels of 0.05–0.4% (0.5–4 mg/ml), producing daily intakes (not measured) which might have been as high as 700–800 mg/kg. His experiments were similarly negative, both in respect to direct mutagenesis and enhancement of mutation by either irradiation (^{60}Co source) or a chemical mutagen (TEPA). It is apparent that the mutagenicity of caffeine, as measured by induction of dominant lethal mutations in mice, is either nonexistent or of such a low order that much larger experiments than those conducted to date would be necessary to demonstrate it.

The dominant-lethal experiments of Kuhlmann *et al.* (1968) showed apparently positive results, but Adler (1970), in her review of studies on caffeine mutagenicity, pointed out a number of deficiencies in Kuhlmann's experiments. Her criticisms included: low numbers of control females, abnormally low incidence of embryonic deaths, control series not strictly parallel, use of inbred strains of mice, and inclusion of females

in which all embryos died. For all these reasons, and in the face of negative data from three other laboratories (Röhrborn, 1968; Epstein *et al.*, 1970; and the present study), the work of Kuhlmann *et al.* (1968) must be regarded as, at least, questionable.

The lack of enhancing effects on mutagenicity is shown in the acute experiment in which caffeine was administered close to the time of irradiation, when it would be most likely, if at all, to inhibit DNA repair mechanisms. The work of Domon and Rauth (1969), Rauth *et al.* (1967, 1970), and Wragg *et al.* (1967) has led some to expect enhancement of mutagenesis when inhibition of DNA repair occurs. Rauth found that caffeine inhibits dark-repair of irradiation damage in mouse cells in culture (measured by colony-forming efficiency). However, this was found only for damage produced by ultraviolet radiation (in the range of 50–400 ergs/mm^2) but not by ionizing radiation (100–800 rads). This in general agrees with earlier bacterial studies. Furthermore, a concentration of at least 0.3 mM (58 μg/ml) was required to produce an effect on recovery from ultraviolet irradiation, and 2 mM (388 μg/ml) was optimal. The minimum level is approximately five times as high as the level that might be expected in testicular tissue of a CD-1 mouse receiving a single dose of 15 mg/kg (Burg and Werner, 1972) and 10 times the maximum human blood concentration observed by Axelrod and Reichenthal (1953). In similar studies by Rauth *et al.* (1970) showing enhancement by caffeine of the cell-killing activity of mitomycin C, only the high (2 mM) concentration of caffeine was used.

The more direct studies of Wragg *et al.* (1967) on DNA polymerase activity in cell-free extracts and on DNA synthesis in cultured cells showed a requirement for activity of concentrations of caffeine of 200 μg/ml or higher. Similarly, the chromosome breakage studies of Ostertag *et al.* (1965) using HeLa cells in culture showed minimal positive effects at 500 μg/ml, the minimum concentration tested. Continuous exposure of HeLa cells to caffeine at levels of 5–20 μg/ml for periods up to 48 cell divisions did not produce any increase in chromosomal abnormalities (Thayer *et al.*, 1971). Recent work has shown increases in abnormalities after 4 weeks exposure with subculturing to concentrations of 40–160 μg/ml (Bishun *et al.*, 1973).

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REFERENCES

ADLER, I. D. (1970). The problem of caffeine mutagenicity. In: *Chemical Mutagenesis in Mammals and Man* (F. Vogel and G. Röhrborn, eds.), pp. 383–403. Springer-Verlag, Berlin.

AXELROD, J. AND REICHENTHAL, J. (1953). The fate of caffeine in man and a method for its estimation in biological material. *J. Pharmacol. Exp. Ther.* **107**, 519–523.

BATEMAN, A. J. (1966). Testing chemicals for mutagenicity in a mammal. *Nature (London)* **210**, 205–206.

BATEMAN, A. J. AND EPSTEIN, S. S. (1971). Dominant lethal mutations in mammals. In: *Chemical Mutagens* (A. Hollaender, ed.), Vol. 2, pp. 541–568. Plenum, New York.

BISHUN, N. P., WILLIAMS, D. C. AND RAVEN, R. W. (1973). Chromosome damage to HeLa cells grown continuously in caffeine. *Mutat. Res.* **17**, 145–146.

BURG, A. W. AND WERNER, E. (1972). Tissue distribution of caffeine and its metabolites in the mouse. *Biochem. Pharmacol.* **21**, 923–937.

DOMON, M. AND RAUTH, A. M. (1969). Ultraviolet-light irradiation of mouse L cells: Effects on cells in the DNA synthesis phase. *Radiat. Res.* **40**, 414–429.

EPSTEIN, S. S., BASS, W., ARNOLD, E. AND BISHOP, Y. (1970). The failure of caffeine to induce mutagenic effects or to synergize the effects of known mutagens in mice. *Food Cosmet. Toxicol.* **8**, 381–401.

KUHLMANN, W., FROMME, H. G., HEEGE, E. M. AND OSTERTAG, W. (1968). The mutagenic action of caffeine in higher organisms. *Cancer Res.* **28**, 2375–2389.

OSTERTAG, W., DUISBERG, E. AND STURMANN, M. (1965). The mutagenic activity of caffeine in man. *Mutat. Res.* **2**, 293–296.

RAUTH, A. M. (1967). Evidence for dark-reactivation of ultraviolet light damage in mouse L-cells. *Radiat. Res.* **31**, 121–138.

RAUTH, A. M., BARTON, B. AND LEE, C. P. Y. (1970). Effects of caffeine on L-cells exposed to mitomycin C. *Cancer Res.* **30**, 2724–2729.

RÖHRBORN, G. (1968). Mutagenicity tests in mice. I. The dominant lethal method and the control problem. *Humangenetik* **6**, 345–361.

THAYER, P. S., HIMMELFARB, P., LISS, R. H. AND CARLSON, B. L. (1971). Continuous exposure of HeLa cells to caffeine. *Mutat. Res.* **12**, 197–203.

WRAGG, J. B., CARR, J. V. AND ROSS, V. C. (1967). Inhibition of DNA polymerase activity by caffeine in a mammalian cell line. *J. Cell Biol.* **35**, 146A–147A.

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Exposure of Four Generations of Mice to Caffeine in Drinking Water

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Exposure of Four Generations of Mice to Caffeine in Drinking Water. THAYER, P. S. AND KENSLER, C. J. (1973). *Toxicol. Appl. Pharmacol.* **25**, 169–179. A population of CD-1 mice has been bred for four generations with continuous ingestion of caffeine. Three caffeine levels (and a control) were used with intakes averaging 4–5, 12–18 and 25–39 mg/kg/day (25–39 mg/kg/day is equivalent to 19–30 cups of coffee in man). At each level 20 pairs of animals were bred in each generation, the F_1 matings being divided between brother-sister matings and out-crosses. F_2's and F_3's were out-crossed. There were no consistent, dose-related effects of caffeine on fertility, age at sexual maturity, mean litter size, weight of offspring at weaning, sex ratio or fetal abnormalities. A few pairs at the highest caffeine level failed to produce litters, but only in the F_1 brother-sister and in the F_3 matings. Litters in the caffeine-treated series showed a somewhat higher incidence of underweight animals in the F_2 and the F_3 generations, but not in a dose-related way. A difference in sex ratios (males slightly predominating) in the caffeine-treated groups compared to the control groups is related to an apparent deficit of male births in the control groups rather than to an effect of caffeine. In this study 3824 caffeine-exposed offspring (352 litters) were examined at birth for birth defects and 1365 of these (147 litters) were reexamined at weaning age. The only defect observed was in one litter (of F_2 offspring from out-cross parents receiving the middle dose of caffeine) with a digital abnormality. This low frequency of abnormal offspring is consistent with control group experience. Further litters from the same parental pair continued on caffeine showed no abnormalities. These studies in the mouse, thus support the safety of caffeine in relatively high doses during reproduction.

In the last several years, a large number of studies on the effects of caffeine on microorganisms, plants, cells in culture, fruit flies and mammalian genetic systems have been reported (Röhrborn, 1968; Epstein *et al.*, 1970; Thayer and Kensler, 1973). However, no reproduction study of the effects of ingested caffeine with more than two generations of a mammalian species has been reported. In a recent review of genetic and reproductive studies related to caffeine (Adler, 1970), only one such paper is cited. Eichler and Mügge (1932) injected male and female rats sc before and during the mating period with 100 mg/kg of caffeine per day for four generations. These studies showed neither mutagenic nor teratogenic effects.

Other studies of the effects of caffeine ingestion on reproduction and growth have dealt with at most one offspring generation and have variously shown with high doses of

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caffeine: reduction of male mouse fertility (Cattanach, 1962); decreased reproductive capacity of mated pairs of mice (Lyon *et al.*, 1962); no effects on reproduction or growth of rats (Bachmann *et al.*, 1946); some effects on rat weanling growth but little or none on reproduction (Hamill and Miller, 1935); and "some evidence of reduced fecundity" in rats (Cheney, 1944). The effect level of caffeine in these studies ranged from 5 mg/kg and higher. Similarly, work in this laboratory (Thayer and Kensler, 1973) showed reduced fertility in male mice after 8 weeks' ingestion of caffeine at 49 and 122 mg/kg, but not at 13 mg/kg. There have been few reports of teratogenic investigations with caffeine, most notable being those of Bertrand *et al.* (1965, 1969, 1970), Nishimura and Nakai (1960), Fujii and Nishimura (1969), Fujii *et al.* (1969), and Snigorska and Bartel (1970), all of whom used large acute doses of caffeine administered parenterally. Positive results were obtained at doses of 50 mg/kg or higher.

The objectives of the present study were to expose several generations of mice to continuous caffeine ingestion, and to determine whether caffeine had effects on fertility, litter size, growth rate of offspring, sex ratio and incidence of gross teratologic abnormalities.

Caffeine was administered by the oral route at levels comparable to and above the range of human ingestion of caffeine (on a mg/kg basis). The levels chosen resulted in caffeine intakes of approximately 4–5, 12–18 and 25–39 mg/kg/day. For a 70-kg person these would require approximate intakes of 3–4, 10–14, and 20–31 cups of coffee daily, thereby representing normal to very high intake.

METHODS

Caffeine solutions were prepared in tap water weekly, or as needed. Caffeine (mp 239°C) was obtained from Mann Research Laboratories. The concentrations were 0.02, 0.06, and 0.12 mg/ml. All caffeine-treated animals received caffeine in their drinking water.

Animals. This experiment was conducted with CD-1 mice obtained from Charles River Laboratories and their offspring. Mated animals were housed in individual cages and were not separated either before or after the birth of a litter. They were fed Purina Laboratory Chow supplied ad libitum.

Breeding schedule. Each experimental group in the first (P_1) generation consisted of 20 females and 10 males. For 4 weeks prior to mating they were housed in stock cages separated by sex and were supplied drinking water containing caffeine at the test levels; and plain tap water was supplied to the controls. Liquid consumption was monitored throughout the premating period. In this generation only, one male was mated with two females, the 10 males being taken at random from a group of 19–20 which had received the pretreatment. In each subsequent generation, each pair (one male and one female per cage) was allowed to produce two litters, the first of which was raised to weaning and the second, sacrificed immediately after birth, used for determination of litter size and examination for birth defects. F_1 offspring were selected for two types of matings. For brother-sister matings, pairs of animals were taken from cages in which only one of the females had produced a litter, and for out-crosses males and females were taken from separate cages. After the birth of second litters to the brother-sister F_1 pairs this line of breeding was discontinued and the two subsequent generations were

continued as out-cross matings. The pairs of F_2 animals were made up in such a way that the male and female had no P_1 ancestors in common. Matings of their offspring, the F_3 pairs, were selected to have no F_1 parents in common but descended in almost all cases from common P_1 ancestors.

Caffeine consumption was measured for the F_2 and F_3 mated pairs during the first week after their caging. The liquid consumption figures were thus based on the total intake of both animals and were averaged for all 20 cages in each test or control group. All treated groups received caffeine continuously through pregnancy, lactation, growth and after weaning throughout all generations. For gross pathologic examination an autopsy was performed on all parents after the weaning of the first litter or birth of the second litter, whichever occurred later. They were given a general examination for gross abnormalities and were specifically examined for stomach ulcers and tumors of the bladder. In addition, an autopsy was performed on the larger numbers of weanlings not used for breeding purposes, particularly in the F_3 and F_4 generations. Examination for teratologic abnormalities was initially made within a day or two after birth, and all retained offspring were carefully checked again at weaning.

RESULTS

No progressive generation changes were observed in any of the parameters measured. The data for each parameter are presented for all generations in a series of combined tables.

Caffeine Consumption

Caffeine consumption figures for the three generations in which this was determined are given in Table 1. For the P_1 generation these are for the 4-week period prior to mating, and in the F_2 and F_3 generations for the first week after pairing. There is an indication of somewhat increased consumption in the later generations.

Reproductive Performance

Reproductive performance is summarized in Table 2, including the number of successful matings for each group and the mean litter sizes for each group. The results for the P_1 generation are for first litters only, since these matings were not continued after obtaining enough F_1's for further breeding. When the overall data are examined for effects of caffeine on fertility as indicated by numbers of litters born, no consistent dose-response relationship is seen. Inhibition of fertility is suggested in the F_1 matings of brothers and sisters but not in out-cross matings, is not dose-related in the matings of F_2's and is only suggested in the F_3 matings. The latter would appear to be explained by the presence of 6 infertile pairs. The females from five of the six were subsequently found to be fertile when mated with males from other cages in the same caffeine group. Since only scattered instances of inability to carry a litter to birth were seen, a gestation index was not calculated. Similarly, stillbirths were infrequently noted and randomly distributed among control and treated groups and in all generations.

Litter sizes do not appear to be related to caffeine concentration. In the cases in which average first litter size appeared to be somewhat smaller for a caffeine group than the control group, this difference disappeared for the second litters (e.g., F_1 out-cross at

TABLE 1

CAFFEINE CONSUMPTION AND BODY WEIGHT OF MICE

Generation	Animals		Duration of measurement (weeks)	Caffeine concentration (mg/ml)	Mean body wt. (g)		Mean consumption	
	Sex	N			Initial	Final	ml/day	mg/kg/day
P_1	♀	20	4	0	22.5	27.0	5.3	0
		20	4	0.02	22.1	28.1	5.1	4.2
		20	4	0.06	20.8	27.9	4.9	12.2
		20	4	0.12	21.4	27.2	5.5	27.6
	♂	19	4	0	33.3	37.7	7.4	0
		20	4	0.02	33.7	37.4	7.4	4.1
		20	4	0.06	33.8	37.6	7.3	12.2
		20	4	0.12	33.0	37.3	7.5	25.2
						♂ + ♀		
F_2	Pair	20	1	0	59.6	(31.2 + 28.4)	14.9	0
		20	1	0.02	55.8	(29.2 + 26.6)	14.0	5.0 ± 0.7
		20	1	0.06	61.0	(32.3 + 28.7)	14.3	14.0 ± 2.1
		20	1	0.12	58.3	(30.4 + 27.9)	18.9	39.1 ± 3.8
F_3	Pair	20	1	0	68.7	(35.6 + 33.1)	17.1	0
		20	1	0.02	68.8	(33.8 + 35.0)	17.2	5.0 ± 0.5
		20	1	0.06	72.7	(35.7 + 37.0)	21.6	17.8 ± 2.0
		20	1	0.12	62.3	(31.1 + 31.2)	17.5	33.7 ± 4.0

TABLE 2

REPRODUCTIVE RATE AND LITTER SIZES IN MICE AFTER CAFFEINE ADMINISTRATION

Mated generation	Caffeine concentration (mg/ml)	N♀	First litters			Second litter		
			N	Births	Mean	N	Births	Mean
P_1	0	20	18	162	9.0			
	0.02	20	17	147	8.6			
	0.06	20	11	104	9.4			
	0.12	20	14	117	8.4			
F_1 (brother-sister)	0	10	10	91	9.1 ± 1.9	8	75	9.5 ± 1.2
	0.02	10	9	99	11.0 ± 2.1	9	105	11.7 ± 2.2
	0.06	10	9	93	10.3 ± 1.2	8[b]	100	12.5 ± 3.5
	0.12	10	7	66	9.4 ± 2.1	4[a,b]	40	10.0 ± 2.6
F_1 (out-cross)	0	10	10	105	10.5 ± 3.3	10	115	11.5 ± 3.0
	0.02	10	9	88	9.8 ± 3.2	9	109	12.1 ± 3.7
	0.06	10	10	89	8.9 ± 2.0	9	117	13.0 ± 1.3
	0.12	10	9	91	10.1 ± 1.3	8	92	11.5 ± 2.6
F_2	0	20	20	218	10.9 ± 1.7	20	249	12.5 ± 3.2
	0.02	20	16	138	8.6 ± 2.5	13[a]	162	12.5 ± 1.7
	0.06	20	17	194	11.4 ± 1.9	14[a]	174	12.4 ± 3.9
	0.12	20	20	205	10.3 ± 2.7	20	248	12.4 ± 3.7
F_3	0	20	19[c]	201	10.6 ± 2.9	19	241	12.7 ± 3.2
	0.02	20	18[c]	188	10.4 ± 2.0	18	207	11.5 ± 2.6
	0.06	20	19	200	10.5 ± 2.0	19	263	13.8 ± 2.5
	0.12	20	14[c]	151	10.8 ± 2.0	12[b]	151	12.6 ± 3.1
	0.12[d]	20	19	201	10.6 ± 2.4	17	187	11.0 ± 3.9

[a] Accident or death before second litter.
[b] Female sacrificed before second litter.
[c] Some infertile pairs: Females retested—all but one fertile. One in 0 mg/ml, two in 0.02 mg/ml, five in 0.12 mg/ml. No offspring from one female in the 0.12 mg/ml concentration of caffeine.
[d] Including litters sired by second male.

0.06 mg/ml and F_2 at 0.02 mg/ml). Thus, the sporadic incidence of lower pregnancy rates is more likely a generalized effect on fertility or mating behavior especially with primiparous females, than a specific effect on gametes or embryos, e.g., dominant-lethal mutation.

Another criterion of reproductive success is the ability of the female to raise offspring to weaning age (5 weeks). The percentage of offspring raised to weaning age ranged between 80 and 97% with no consistent dose-related effect of caffeine (Table 3). Control values ranged between 80 and 93%. There are three cases in which the weaning rate appears lower: at the 0.06 mg/ml level for the F_1 brother-sister matings, and at 0.06 and 0.12 mg/ml for the F_2 matings. However, in the first case, two litters were lost through laboratory accidents; in the F_2 mating at 0.06 mg/ml there was one cage in which the female and all F_3 offspring were killed by the male and another in which the female died of an unknown cause. The low values for the F_3 offspring at the 0.12 mg/ml level are accounted for in large part by deaths in large litters, five of them with birth numbers of 11 or larger, accounting for 64 births and 44 of the 58 deaths observed.

TABLE 3

WEANING PERCENTAGES, SEXES AND WEIGHTS, IN MICE AFTER CAFFEINE ADMINISTRATION

Generation	Caffeine concentration (mg/ml)	No. born	Total weaned		No. of litters	Mean weight at weaning			
						♂		♀	
			N	%		N	g	N	g
F_2 (B/S)	0	91	75	82	10	28	24.5 ± 3.0	47	20.4 ± 3.1
	0.02	99	91	92	9	37	20.7 ± 5.2	54	20.3 ± 4.4
	0.06	93 (72)[a]	69	74 (96)	7	32	24.6 ± 4.0	37	20.6 ± 4.4
	0.12	66 (59)[b]	58	88 (98)	6	29	24.2 ± 3.8	29	21.2 ± 2.9
F_2 (out-cross)	0	105	98	93	10	44	25.1 ± 4.8	54	22.2 ± 2.3
	0.02	88	78	89	9	41	23.9 ± 5.4	37	20.2 ± 3.4
	0.06	89	86	97	10	41	23.5 ± 4.2	45	21.7 ± 4.3
	0.12	91	86	94	9	49	24.1 ± 3.7	37	21.2 ± 3.2
F_3	0	218 (209)[b]	174	80 (83)	19	71	23.1 ± 4.0	103	19.8 ± 3.7
	0.02	138	130	94	16	62	24.3 ± 3.8	68	20.4 ± 5.1
	0.06	194 (169)[c]	149	77 (88)	14	84	22.2 ± 4.5	65	20.4 ± 3.2
	0.12	205 (198)[b]	147	72 (74)	19	71	23.8 ± 6.1	76	19.8 ± 4.6
F_4	0	201 (185)[d]	175	87 (95)	17	95	22.5 ± 4.2	80	18.9 ± 4.1
	0.02	188 (178)[b]	152	81 (85)	17	75	22.5 ± 3.1	77	19.7 ± 2.8
	0.06	200	189	95	19	111	23.6 ± 3.2	78	20.3 ± 2.3
	0.12	151 (141)[b]	130	86 (92)	13	62	23.6 ± 3.5	68	17.8 ± 3.3

[a] Two litters lost through laboratory accidents.
[b] Cannibalism occurred on days 1–3 in one litter.
[c] Cannibalism occurred in two litters. One female killed by male, offspring died.
[d] Cannibalism occurred in two litters.

TABLE 5

SEX DISTRIBUTION AT WEANING IN MICE AFTER CAFFEINE ADMINISTRATION

Offspring generation	Caffeine concentration (mg/ml) 0		0.02		0.06		0.12		All caffeine	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
F_2-B/S	28	47	37	54	32	37	29	29	98	120
									$R = 0.82^a$	
F_2 (out-cross)	44	54	41	37	41	45	49	37	131	119
							(+1 litter)		$R = 1.10$	
F_3	71	103	62	68	84	65	71	76	217	209
									$R = 1.04$	
F_4	95	80	75	77	111	78	62	68	248	223
									$R = 1.11$	
Total (all generations)	238	284	215	236	268	225	211	210		
R	0.84		0.91		1.19		1.00			
					694♂	671♀				
					$R = 1.03$					

[a] R = males/females.

Female Age

As a possible source of differences in reproductive success we have examined the mean age of females at the time of mating and the length of time until the birth of their first litter. These data are presented for each generation and each caffeine level in Table 6. The F_2 and F_3 parents appear to show somewhat earlier births of first litters compared to the F_1's. However, the F_1 animals may not have reached sexual maturity at the time of mating, as it can be seen that the ages of the F_1 and F_2 females at parturition (the sums of the two values given) are very nearly the same. The F_2 and F_3 females were older at the time of mating because no pairings were made until sufficient animals were available for the whole group. The larger SD for time to first litter in several groups —namely, F_1 brother-sister, controls and 0.12 mg/ml caffeine; F_1 out-cross, 0.06 and 0.12 mg/ml caffeine; and F_3, controls and 0.12 mg/ml caffeine—are each attributable to the presence of a few pairs in which successful mating was delayed considerably beyond the rest of the group. No consistent or dose-related effects of caffeine are evident in these data.

Birth Defects

The numbers of animals born and weaned in this study are summarized in Table 7. A total of 3824 offspring (first and second litters included) were born of caffeine-treated parents, and all these were examined at birth for gross birth defects, particularly those of the limbs. Since only the first litter of any pair was raised to weaning age, the number of animals examined for defects at weaning ranged from 400 to 500 in each dose group.

TABLE 6

FEMALE AGE AT MATING AND TIME TO FIRST LITTER IN MICE AFTER CAFFEINE ADMINISTRATION

Generation mated	Caffeine concentration (mg/ml)	N	Age at mating (days)	Time to first litter (days)
F_1-(B/S)	0	10	40.4 ± 1.0	42.0 ± 22.9
	0.02	9	40.6 ± 0.5	29.6 ± 2.4
	0.06	9	41.4 ± 0.5	30.2 ± 8.8
	0.12	7	41.3 ± 0.5	36.0 ± 11.4
F_1 (out-cross)	0	10	40.5 ± 1.1	33.1 ± 7.5
	0.02	9	40.3 ± 1.0	26.9 ± 9.1
	0.06	10	41.0 ± 0	40.8 ± 24.4
	0.12	10[a]	39.8 ± 3.1	43.3 ± 34.2
F_2	0	20	47.3 ± 5.3	27.2 ± 7.1
	0.02	17[a]	53.7 ± 4.1	24.8 ± 7.6
	0.06	17	58.3 ± 16.3	27.9 ± 7.6
	0.12	20	50.4 ± 5.3	24.1 ± 5.6
F_3	0	19	79.5 ± 6.3	30.1 ± 13.6
	0.02	18	84.1 ± 9.3	25.5 ± 6.3
	0.06	19	70.5 ± 6.4	21.7 ± 1.7
	0.12	14	84.8 ± 6.6	29.2 ± 13.4

[a] Includes one litter not raised to weaning age.

TABLE 7

ANIMALS EXAMINED FOR GROSS EXTERNAL ABNORMALITIES AFTER CAFFEINE ADMINISTRATION[a]

Caffeine concentration (mg/ml)	At birth				At weaning (1st litters only)	
	1st litters		2nd litters			
	Litters	Individuals	Litters	Individuals	Litters	Individuals
0	77	777	57	680	66	522
0.02	69	660	49	583	51	451
0.06	66	680	50	654	50	493
0.12	69	680	49	567	46	421
All caffeine	204	2020	148	1804	147	1365

[a] All generations pooled.

Only one litter appeared which showed a possible birth defect, and this appears to be a chance occurrence. The mating in question was an F_1 pair (out-cross) receiving caffeine at 0.06 mg/ml. Their first litter included seven males and one female, each of which was missing some toes on one or more feet. There was no common pattern as to which foot had missing toes, nor as to how many toes were missing, suggesting that this might have been attributable to a mild form of cannibalism rather than a true birth defect. Even

though there is the possibility that such a minor defect could have been missed at birth, the fact remains that it was not noted. The same pair had three more litters while continuing to receive caffeine. These litters, with 11, 12, and 7 births, respectively, showed no incidence of the toeless condition. The female member of the original F_2 litter died before it was possible to breed her. However, six of the males were bred, four of them to other females from the 0.06 mg/ml caffeine group (continued on caffeine) and two of them (removed from caffeine) to proved females from the stock colony. All these matings resulted in litters ranging in size from 8 to 15, none of which showed any incidence of digital abnormality. Thus, several factors would appear to minimize the likelihood of the abnormality being attributable to a direct effect of caffeine: it occurred at the middle dose of caffeine, the male and female (maintained on caffeine) involved produced no further such litters, there were a large number of births observed without such abnormalities, and this strain of mouse is known to show a low percentage of birth defects (personal communication, Dr. Henry Foster, Charles River Laboratories).

Gross Pathology

As indicated above, an autopsy was performed on all breeding animals and they were examined for gross internal pathology, including in particular, gastric ulcers (starting with the F_1 breeders) and bladder abnormalities (starting with the F_2 breeders). In addition, certain groups of animals were held for longer periods of time for examination for bladder defects. In particular, 71 F_3 animals distributed among the 3 caffeine groups were held on caffeine for autopsy at an age of 8 months. Animals of the parental groups were 3–4 months old at the time of sacrifice. In none of these animals were any stomach (736 animals) or bladder (358 animals) abnormalities seen.

Most animals, of course, were not kept long enough for any significant incidence of tumors to be expected unless one were dealing with a potent carcinogen. The only tumor seen in the study was an abdominal mass found in an F_3 breeding female on 0.06 mg/ml caffeine at the age of 105 days. This animal also exhibited splenomegaly. We attach no significance to this isolated observation.

DISCUSSION

The breeding behavior of the control population for four generations in these experiments was well within normal expectations. This includes the criteria of pregnancy rates, litter sizes in first and second litters, weaning success, weights at weaning, and absence of anatomical abnormality. None of these parameters was significantly affected in a dose-related way by continuous intake of caffeine although underweight weanlings and infertile matings were sporadically observed in different generations and at different levels of caffeine. Neither these, nor any other possible manifestations of caffeine ingestion showed any consistent dose-related pattern of occurrence, nor was there any evidence of progressive change from generation to generation. Experiments done in this laboratory on metabolism of caffeine in CD-1 mice from the same stock (Burg and Werner, 1972) have demonstrated that caffeine is equilibrated through all tissues and is rapidly excreted from the body (plasma half-time, 3 hr or less). Furthermore, animals from the F_3 generation of the present study did not show any changes, compared to previously unexposed animals, in several measures of the ability to metabolize caffeine.

This included tissue distribution of [^{14}C]-caffeine as demonstrated by whole-body radioautography in pregnant animals, and rate and amounts of caffeine and metabolites excreted in expired air, feces, and urine (R. H. Liss and J. P. Schepis, unpublished observation).

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REFERENCES

ADLER, I. D. (1970). The problem of caffeine mutagenicity. In: *Chemical Mutagenesis in Mammals and Man* (F. Vogel and G. Röhrborn, eds.), pp. 383–403. Springer-Verlag, Berlin.

BACHMANN, G., HALDJ, J., WYNN, W. AND ENSOR, C. (1946). Reproductivity and growth of albino rats on a prolonged daily intake of caffeine. *J. Nutr.* **32**, 239–247.

BERTRAND, M., member of the Group for the Study of Teratogenic Risks (1969). Teratogénèse expérimentale: Etude de la Caféine chez la Souris. *Thérapie* **24**, 575–580.

BERTRAND, M., SCHWAM, E., FRANDON, A., VAGNE, A. AND ALARY, J. (1965). Sur un effet tératogène systématique et spécifique de la caféine chez les Rongeurs. *C. R. Soc. Biol.* **159**, 2199–2202.

BERTRAND, M., GIROD, J. AND RIGAUD, M. F. (1970). Ectrodactylie provoquée par la caféine chez les Rongeurs. Rôle des facteurs spécifiques et génétiques. *C. R. Soc. Biol.* **164**, 1488–1489.

BURG, A. W. AND WERNER, E. (1972). Tissue distribution of caffeine and its metabolites in the mouse. *Biochem. Pharmacol.* **21**, 923–937.

CATTANACH, B. M. (1962). General effects of caffeine in mice. *Z. Vererbungslehre* **93**, 215–219.

CHENEY, R. H. (1944). Variation in reproductive phenomena by caffeine. *Fed. Proc., Fed. Amer. Soc. Exp. Biol.* 3, 68 (Abstract).

EICHLER, O. AND MÜGGE, H. (1932). Zur Frage der Schädlichkeit des Coffeins bei chronischer Zufuhr. *Arch. Exp. Pathol. Pharmakol.* **168**, 89–96.

EPSTEIN, S. S., BASS, W., ARNOLD, E. AND BISHOP, Y. (1970). The failure of caffeine to induce mutagenic effects or to synergize the effects of known mutagens in mice. *Food Cosmet. Toxicol.* **8**, 381–401.

FUJII, T. AND NISHIMURA, H. (1969). Teratogenic actions of some methylated xanthines in mice. *Okajimas Folia Anat. Jap.* **46**(4), 167–175.

FUJII, T., SASAKI, H. AND NISHIMURA, H. (1969). Teratogenicity of caffeine in mice related to its mode of administration. *Jap. J. Pharmacol.* **19**, 134–138.

HAMILL, H. H. AND MILLER, L. A. (1935). The effect of caffeine containing beverages upon growth, reproduction and lactation of albino rats. *Proc. Okla. Acad. Sci.* **15**, 63–65.

LYON, M. F., PHILLIPS, R. J. S. AND SEARLE, A. G. (1962). A test for mutagenicity of caffeine in mice. *Z. Vererbungslehre* **93**, 7–13.

NISHIMURA, H. AND NAKAI, K. (1960). Congenital malformations in offspring of mice treated with caffeine. *Proc. Soc. Exp. Biol. Med.* **104**, 140–142.

RÖHRBORN, G. (1968). Mutagenicity tests in mice. I. The dominant lethal method and the control problem. *Humangenetik* **6**, 345–361.

SNIGORSKA, B. AND BARTEL, H. (1970). Badania Nad Teratogennym Wplywem Kofeiny Na Plody Myszy Bialej. *Folia Morphol. (Warsaw)* **29**, 353–363.

THAYER, P. AND KENSLER, C. J. (1973). Genetic tests in mice of caffeine alone and in combination with mutagens. *Toxicol. Appl. Pharmacol.* **25**, 157–168.

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CAFFEINE AND BLADDER CANCER

SIR,—Dr. Cole, in his interesting paper [1] showing an apparent association between coffee-drinking and bladder cancer, suggests that caffeine may be involved. This seems a priori unlikely if only because his non-coffee-drinking controls—unless they restrict themselves to water, selected soft drinks, and alcohol—will have at least some caffeine intake, since caffeine is present not only in coffee but also in tea, cocoa, and 'Coca-Cola'. While caffeine is at lower concentrations in these beverages, there must be considerable overlap in the total caffeine intake of coffee-drinkers and non-coffee-drinkers. In addition, although it is true that caffeine has been shown to be mutagenic in lower organisms, in recent reviews there was no definite evidence for caffeine mutagenicity in mice [2] or man.[3] Indeed, caffeine is probably the most intensively investigated potential human mutagen for which at present no clear mutagenic activity in mammals has been established.[4]

An interesting feature of Dr. Cole's data is the difference in the relative risks of bladder cancer between males and females among coffee-drinkers when controlled for age, cigarette-smoking, and occupation. Relative to a risk of 1·00 among non-coffee-drinkers he found the risk to men coffee-drinkers to be 1·24 and to women coffee-drinkers 2·58. It has been shown [5] that caffeine at a concentration of $10^{-4}M$ (equivalent to 8 cups of coffee or 11 cups of tea) stimulates the rate of mitosis of human lymphocytes from female donors in vitro, while at the same concentration it decreases the mitotic rate of lymphocytes from male donors when compared with control cultures from the same individuals containing no caffeine. If caffeine has the same effect on the mitotic rate of bladder-cancer cells, then this might explain the difference found by Dr. Cole between the sexes.

1. Cole, P. *Lancet*, 1971, i, 1335.
2. Epstein, S. *in* Chemical Mutagenesis in Mammals and Man (edited by F. Vogel and G. Rohrborn); p. 404. Berlin, 1970.
3. Vogel, F. *ibid.* p. 433.
4. Adler, I. D. *ibid.* p. 383.
5. Timson, J. *Br. J. Pharmac.* 1970, 38, 731.

It seems, therefore, reasonable to suggest that, while coffee may well contain carcinogens active in the bladder, the caffeine in the coffee (or from other sources) has a secondary effect on the incidence of bladder cancer by its action on the rate of mitosis of the malignant cells. This would mean that caffeine would stimulate the growth of the cancer in women and retard it in men, leading to an apparent higher incidence of coffee-drinkers in female patients with bladder cancer.

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EFFECT OF THEOBROMINE, THEOPHYLLINE AND CAFFEINE ON THE MITOSIS OF HUMAN LYMPHOCYTES

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SUMMARY

The effect of theobromine, theophylline and caffeine on the mitosis of human peripheral blood lymphocytes in short-term culture has been investigated. The results show that at high concentrations all three plant alkaloids are antimitotic or cytostatic. At lower concentrations theophylline is more antimitotic than caffeine while theobromine is without effect on mitosis. The effect of caffeine at lower concentrations is related to the sex of the lymphocyte donor. This is not the case with theophylline or theobromine. The results are discussed in relation to the chemical structures of these compounds and their consumption by man. It is suggested that the antimitotic effects are related to the metabolic products of these compounds in man and that uric acid derivatives, especially when methylated in position 1, are strongly antimitotic. The apparent lack of mutagenic activity of these compounds in man may be due to the antimitotic threshold being the same as the mutagenic threshold so that any mutant cells produced are unable to reproduce and that therefore these compounds are effectively nonmutagenic in man.

INTRODUCTION

The three plant alkaloids, theobromine (3,7-dimethylxanthine), theophylline (1,3-dimethylxanthine) and caffeine (1,3,7-trimethylxanthine) are widely distributed in plants. Most importantly for man they are found in the leaves of plants used in the manufacture of many widely consumed drinks. Coffee, maté and the cola drinks contain caffeine; tea contains caffeine and theophylline; and the cocoa drinks caffeine and theobromine. It is only possible to give approximate figures for the amount of these compounds found in various drinks since this will vary according to the method of preparation. A cup of coffee contains 95–125 mg caffeine; a cup of tea 60–90 mg of caffeine; and a cup of cocoa 30–50 mg caffeine plus 95–150 mg theobromine. A 360-ml bottle of coca-cola contains 35–55 mg of caffeine. It is reasonable, therefore, to assume that nearly everyone is exposed at least to some extent to one or more of these compounds.

All three have similar pharmacological properties. In particular they stimulate the central nervous system, act on the kidney causing diuresis, stimulate cardiac muscle and relax smooth muscle especially bronchial muscle. Theophylline is regarded as a valuable drug in the emergency treatment of congestive heart failure[10]. Tolerance may develop especially to the diuretic effect in habitual heavy users and cross-tolerance has been known to occur. The fatal dose of caffeine in man is estimated to be about 10 g but deaths have not been recorded. 1 g may be sufficient to cause insomnia[10]. Caffeine withdrawal can cause headaches in susceptible individuals and is an empirical component of some headache remedies[6]. Theophylline is more toxic and deaths from this compound have been recorded[10]. All are readily absorbed into the body by the oral and all other routes. Caffeine has a half-life in the body of about 3.5 h and hence there is no appreciable overnight accumulation[8]. All three compounds are actively metabolised in man with very little, perhaps 1% of caffeine, being excreted unchanged in the urine[3]. Human volunteers taking 1 g of the compound being tested while on a low purine diet excreted within 48 h in the urine as methylxanthines or methyluric acids 62% of the theobromine, 77% of the theophylline, and 66% of the caffeine[4]. Caffeine has been shown to reach the gonads, cross the placental barrier[9] and to be transmitted *via* milk to infants[1].

All these compounds have been shown to be mutagenic in *E. coli*[5,14] and caffeine and theophylline in Ophiostoma[6]. Caffeine is lethal at 1% concentration in plant cells and antimitotic at lower concentrations. All three suppress cell wall formation in Allium at a concentration of 0.02% (ref. 11). All three have been shown to produce chromosome breaks in HeLa cells[15] but experiments *in vivo* with rabbits gave no clear indication of caffeine mutagenicity[16,17] and similar results were obtained with mice[12]. Recently, EPSTEIN[7] has shown that caffeine fails to induce mutagenic effects or to be synergistic with known mutagens in mice. It is, therefore, clear that while man is regularly and continuously exposed to these compounds which have been demonstrated to be mutagenic in lower organisms, there is little or no evidence of mutagenic activity *in vivo* in mammals.

MATERIALS AND METHODS

Blood samples were obtained from healthy adult volunteers. No attempt was made to reduce the normal coffee, tea, *etc.*, intake of the volunteers before the samples were taken since the intention was to study the effects of these compounds on lymphocytes which had been exposed to the usual caffeine, theobromine and theophylline intake of the volunteers. About 20 ml of venous blood was mixed immediately after collection with anticoagulant (heparin in dextran) in a sterile container. The erythrocytes were allowed to settle at 37° and the supernatant plasma and lymphocytes were drawn off. 1.5 ml of the cell/plasma suspension was made up to 10 ml with TC199 culture medium[13]. This contains no theobromine, theophylline or caffeine. Two drops of reconstituted phytohaemagglutinin P (Difco) were added to each culture at the start of the 72-h culture period at 37°. At the end of this time 0.2 ml of demecolcine ("colcemid", Ciba) at a concentration of 1 mg in 100 ml of TC199 was added to each culture which was then incubated at 37° for a further 2 h. The cells were then spun down, the supernatant was discarded and the cells were resuspended in hypotonic saline for 15 min at 37°. They were then fixed in two changes of acetic alcohol (3 parts

ethanol, 1 part glacial acetic acid by volume) with a minimum period of 1 h in each change, spun down and resuspended in sufficient 45% acetic acid to give an opalescent suspension. The cells were then dropped onto cold slides, air-dried, and stained with 10% Giemsa in pH 6.4 buffer. The experimental cultures were exposed to 10^{-2}, 10^{-3} or 10^{-4} M theobromine, theophylline or caffeine throughout the 72-h period. Control cultures from each blood sample without the test compound were set up at the same time. Since there is considerable variation in the mitotic index between individual donors it is essential to set up an untreated control culture from the same blood sample at the same time using the same batch of culture medium and phytohaemagglutinin as has been done in these experiments. This is particularly important since it has been shown that there is also variation in the mitotic index of cultured lymphocytes taken at different times from a single individual[17]. 1000 cells from each culture were examined and the number of cells in mitosis was recorded. This number is defined as the mitotic index. The averaged results for each series of experiments are given in Table I expressed as a percentage of the control values. These are based on 12 experiments with caffeine, 6 with theophylline and 5 with theobromine.

TABLE I

EFFECT OF THEOBROMINE, THEOPHYLLINE AND CAFFEINE ON THE RATE OF MITOSIS OF HUMAN LYMPHOCYTES IN CULTURE

Concentration (M)	*Mitotic rate (% control)*		
	Theobromine	*Theophylline*	*Caffeine*
10^{-2}	3	0	0
10^{-3}	106	0	41
10^{-4}	98	16	111
0 (control)	100	100	100
Positions of methyl groups	3,7	1,3	1,3,7

DISCUSSION

The results presented here show that theobromine, theophylline and caffeine have cytostatic (no cells in mitosis) and antimitotic effects on human lymphocytes in culture. Chromosome damage, however, was not induced by these compounds in these experiments. Theobromine had the least effect being active only at 10^{-2} M where it was strongly antimitotic but not cytostatic. Caffeine was found to be cytostatic at 10^{-2} M and antimitotic at 10^{-3} M but not at 10^{-4} M. When the results with caffeine at 10^{-4} M were subdivided on the basis of the sex of the lymphocyte donor it was found that female cells averaged 192% of the control while male cells averaged only 68% of the control as previously described[20]. Statistical analysis showed that there was a significant difference between the response of male and female lymphocytes to 10^{-4} M caffeine[20]. Similar sex differences were not found with theophylline or theobromine. Theophylline was the most active of the three compounds being cytostatic at 10^{-2} and 10^{-3} M and strongly antimitotic at 10^{-4} M.

The order of increasing antimitotic activity of these compounds in human lymphocyte cultures is therefore: theobromine, caffeine, theophylline which is consistent with the pharmacological data. Since theobromine is methylated at positions 3 and 7, caffeine at positions 1, 3 and 7, and theophylline at positions 1 and 3, this

data suggests that the methyl group at position 1 is the most active in suppressing mitosis. This is consistent with the data of CORNISH AND CHRISTMAN[4] who showed that in man the order of increasing stability of these methyl groups in these compounds is 3, 7, 1. It may be, therefore, that the antimitotic activities of these compounds reflect the ability of the cells to remove the methyl groups from the xanthine molecule. After administration to human volunteers it was demonstrated that the main excretory products in the urine were as follows. Most of the theobromine was excreted as methylxanthines while the major excretory products of theophylline were methyluric acids. After caffeine approximately equal quantities of methylxanthines and methyluric acids were found in the urine[4]. It is possible, therefore, that the antimitotic effect of theophylline is due to the methyluric acids formed, while the relative lack of antimitotic effect of theobromine suggests that the methylxanthines are much less antimitotic. The intermediate position of caffeine is then consistent with it being converted into a mixture of methylxanthines and methyluric acids. It has been shown[16] that uric acid is strongly antimitotic at 10^{-2} *M* in this system but only slightly antimitotic at 10^{-3} *M*. The same authors found that xanthine is slightly antimitotic at 10^{-2} *M* and stimulates mitosis in cultured human lymphocytes at 10^{-3} and 10^{-4} *M*. The overall picture, therefore, is that uric acid derivatives are antimitotic while xanthine derivatives are much less so, and that methylation at position 1 considerably increases the antimitotic activity. This is supported by the data of CORNISH AND CHRISTMAN[4] who found that the major excretory products after theophylline ingestion were 1-methyluric acid and 1,3-dimethyluric acid. From caffeine both these compounds are produced together with 1-methylxanthine. Theobromine is metabolised mainly to 3-methylxanthine and 7-methylxanthine.

Theobromine, theophylline and caffeine are all potential mutagens in man since they might be incorporated into DNA as base analogues because of their purine nature. This also applies to the monomethylxanthines and the methyluric acids. Any compound which affects the rate of mitosis without killing the cells must also be suspect as a mutagen and this is so in the compounds tested here although at concentrations infrequently achieved in man. However it must be accepted that no mutagenic activity of these compounds, especially caffeine which has been extensively investigated, has been demonstrated in man[1]. COLE[8] has recently suggested that there may be an association between coffee-drinking and cancer of the lower urinary tract but this may be due to a secondary effect in which the rate of growth of the cancer is affected by caffeine[21].

The apparent lack of observable mutagenicity of these compounds in man, therefore, requires explanation. It is of course true that human populations have been exposed to them for several generations and to related compounds such as uric acid for much longer. It is not impossible, therefore, that man has evolved mechanisms to avoid the potential mutagenic effects of this group of compounds. One of these may be the rapid metabolism of these compounds in the body with their consequent short half-life. Human cells may also be particularly sensitive to these compounds in that the threshold for mutagenic activity may be the same as that for mitotic inhibition. Thus the mutant cells produced in the body by these compounds may also be unable to reproduce. This is consistent with the work of OSTERTAG *et al.*[16] who produced an increased rate of chromosome breakage in HeLa cells with a 1-h exposure to caffeine followed by a 24-h recovery period. In the experiments reported here after 72 h

exposure to caffeine no chromosome damage was detected but the mitotic rate was reduced. It is suggested, therefore, that these compounds, especially caffeine and theophylline, are mutagenic in man but that this mutagenicity is not expressed phenotypically or in the offspring since the mutagenic threshold is the same as the antimitotic threshold and therefore the production of a mutant caused by one of these compounds is an extremely rare event. Detection of such a mutation would, therefore, be extremely unlikely and for practical purposes at the levels of consumption of these compounds in the human population, they may be regarded as not effectively mutagenic.

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REFERENCES

1 ADLER, I. D., The problem of caffeine mutagenicity, in F. VOGEL AND G. RÖHRBORN (Eds.), *Chemical Mutagenesis in Mammals and Man*, Springer, Berlin, 1970.
2 AXELROD, J., AND J. REICHENTHAL, The fate of caffeine in man and a method for its estimation in biological material, *J. Pharmacol. Exptl. Therap.*, 107 (1953) 519–523.
3 COLE, P., Coffee-drinking and cancer of the lower urinary tract, *Lancet*, i (1971) 1335–1337.
4 CORNISH, H. H., AND A. A. CHRISTMAN, A study of the metabolism of theobromine, theophylline and caffeine in man, *J. Biol. Chem.*, 228 (1957) 315–323.
5 DEMEREC, M., G. BERTANI AND J. FLINT, A survey of chemicals for mutagenic action in *E. coli*, *Am. Naturalist*, 85 (1951) 119–136.
6 DREISBACH, R. H., AND C. PFEIFFER, Caffeine-withdrawal headache, *J. Lab. Clin. Med.*, 28 (1943) 1212–1219.
7 EPSTEIN, S., The failure of caffeine to induce mutagenic effects or to synergise the effects of known mutagens in mice, in F. VOGEL AND G. RÖHRBORN (Eds.), *Chemical Mutagenesis in Mammals and Man*, Springer, Berlin, 1970.
8 FRIES, N., AND B. KIHLMAN, Fungal mutations obtained with methyl xanthines, *Nature*, 162 (1948) 573.
9 GOLDSTEIN, A., AND R. WARREN, Passage of caffeine into human gonadal and fetal tissue, *Biochem. Pharmacol.*, 11 (1962) 166–168.
10 GOODMAN, L. S., AND A. GILMAN, *The Pharmacological Basis of Therapeutics*, 3rd edition, Macmillan, New York, 1965.
11 KIHLMAN, B., AND A. LAVAN, The cytological effect of caffeine, *Hereditas*, 35 (1949) 109–111.
12 LYON, M. F., J. S. PHILLIPS AND A. G. SEARLE, A test for the mutagenicity of caffeine in mice, *Z. Vererbungslehre*, 93 (1962) 7–13.
13 MORGAN, J. F., H. J. MORTON AND R. C. PARKER, Nutrition of animal cells in tissue culture, I. Initial studies of a synthetic medium, *Proc. Soc. Exptl. Biol. Med.*, 73 (1950) 1–8.
14 NOVICK, A., AND L. SZILARD, Genetic mechanisms in bacteria and bacterial viruses, I. Experiments in spontaneous and chemically induced mutations of bacteria growing in the chemostat, *Cold Spring Harbor Symp. Quant. Biol.*, 16 (1951) 337–343.
15 OSTERTAG, W., E. DUISBERG AND M. STÜRMAN, The mutagenic activity of caffeine in man, *Mutation Res.*, 2 (1965) 293–296.
16 PRICE, D. J., AND J. TIMSON, The effect of oxypurines on the mitotic rate of phytohaemagglutinin stimulated human lymphocytes *in vitro*, *Cytobios*, 4 (1971) 87–91.
17 PRICE, D. J., AND J. TIMSON, Temporal variation in mitotic index of phytohaemagglutinin stimulated human lymphocytes, *Acta Haematol.*, in the press.
18 STIEVE, H., Untersuchungen über die Wechselbeziehung zwischen Gesamtkörper und Keimdrüse, IV. Die Einfluss des Coffeins auf die Fortpflanzung des Russenkaninchens, *Z. Mikrosk.-Anat. Forsch.*, 15 (1928) 599–625.
19 STIEVE, H., Untersuchungen über die Wechselbeziehung zwischen Gesamtkörper und Keimdrüse, VII. Durch Kaffeegenuss bewirkte Schädigung der Hoden und der Fruchtbarkeit, *Z. Mikrosk.-Anat. Forsch.*, 23 (1931) 571–594.
20 TIMSON, J., The effect of caffeine on the mitosis of human lymphocytes in culture, *Brit. J. Pharmacol.*, 38 (1970) 731–734.
21 TIMSON, J., Caffeine and bladder cancer, *Lancet*, ii (1971) 212–213.

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Effects of Decaffeinated and Nondecaffeinated Coffee Ingestion on Blood Glucose and Plasma Radioimmunoreactive Insulin Responses to Rapid Intravenous Infusion of Glucose in Normal Man

By A. WACHMAN, R. S. HATTNER, BARBARA GEORGE AND D. S. BERNSTEIN

Eight normal humans were subjected to paired standard rapid intravenous glucose tolerance tests one hour following the ingestion, on one occasion, of nondecaffeinated and, on a second occasion, of decaffeinated coffee under identical conditions. Nondecaffeinated coffee elevates fasting blood glucose, impairs glucose tolerance as reflected by a lessened fractional blood glucose disappearance rate and relative hyperglycemia, and blunts the insulinogenic response of blood glucose elevation compared to decaffeinated coffee. These effects of nondecaffeinated coffee appeared to be inversely related to the subjects control glucose tolerance. (Metabolism 19: No. 7, July, 539-546, 1970)

RECENT STUDIES[1-4] have shown that the glucose tolerance of humans and other animals may be influenced following the administration of caffeine. Indeed, there is evidence that caffeine may affect glucose metabolism in several ways. Bellet and his colleagues[5-7] have shown that caffeine ingestion can produce a significant rise in serum free fatty acids and catecholamines. Sutherland and his colleagues[8-9] have shown that the inactivation of cyclic 3′, 5′ adenosine monophospate may be inhibited by methyl xanthines by virtue of their inhibition of phosphodiesterase. This study was undertaken in an attempt to elucidate differences in the effects of decaffeinated and nondecaffeinated coffees upon glucose metabolism in man.

MATERIALS AND METHODS

Experimental Design

Eight subjects were utilized (four male and four female). All were normal, nonobese, healthy adults, ages 20–42 (mean age 29 years). For the week prior to testing, each subject was instructed to refrain from ingesting foods containing caffeine or other methyl xanthines.

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Table 1.—Plasma Glucose Values (mg./100 cc.) Following Ingestion of Nondecaffeinated Coffee and Glucose Infusion

Subject	−60	−30	0	10	20	30	40	50	60 min.
1	90	94	94	313	265	250	225	195	175
2	100	103	95	—	258	238	213	200	170
3	70	78	78	288	255	215	188	160	158
4	83	93	90	263	200	173	145	125	115
5	78	93	48	300	253	228	—	198	165
6	93	93	95	248	213	190	168	158	148
7	80	75	80	275	235	208	183	175	163
8	75	85	78	263	230	213	180	165	140
Mean	83.6	89.3	82.3	278.6	238.6	214.4	186.0	172.0	154.3
(SE)	(3.5)	(3.3)	(5.6)	(8.7)	(8.2)	(8.8)	(10.1)	(9.1)	(6.9)

Each subject was tested on the morning following an overnight fast. The subject was given 10 Gm. of decaffeinated instant coffee (Sanka) dissolved in 250 cc. of 100° C distilled water without sweetening agents or milk products, and allowed ten minutes to drink the coffee. A fasting blood sugar was drawn immediately following the ingestion of coffee and at 30 and 60 minutes thereafter. These are designated at -60, -30, and 0-minute samples, respectively. Immediately after the 0-minute sample the subject was rapidly infused intravenously with 0.5 Gm. of glucose per kilogram body weight in 100 cc. of 0.9 per cent NaCl in water over a 2–4 minute period. Blood was obtained at ten minute intervals for one hour following the infusion. Two to five days subsequent to the initial test the test was repeated using 10 Gm. of the nondecaffeinated form of the same instant coffee (Maxwell House) under identical conditions. In two subjects (5 and 6) the order of presentation of decaffeinated and nondecaffeinated coffee was reversed.

Analytical Methods

The caffeine content of the coffees was determined in triplicate by the method of Axelrod and Reichenthal.[10] Blood glucose was estimated in duplicate by Autoanalyzer. Radioimmunoreactive plasma insulin assays were obtained in duplicate by the double antibody method of Soeldner and Slone.[11]

Mathematical and Statistical Methods

Blood glucose fractional disappearance rates (K) were estimated by calculating the slope of a logarithmic regression line fitted to the 10-, 20-, 30-, 40-, 50-, and 60-minute blood glucose values by the method of least squares.[12] The group means for K, blood glucose, and plasma insulin following decaffeinated versus non-decaffeinated coffee were compared by a T test for correlated means at each point in time.[13] The -60-minute versus -40-minute blood glucose means were compared using the same T test for decaffeinated and for nondecaffeinated coffees. A Pearson product-moment correlation coefficient was computed for the differences of the K's between the decaffeinated and nondecaffeinated coffees, versus the K from the decaffeinated coffee, and tested for significance.[13] The pre-glucose insulin values of each subject were similarly compared for the two studies.

RESULTS

Blood Glucose Levels and K Values (Tables 1,2,3, Fig. 1)

No significant difference was noted between the −60-minute blood glucose means between nondecaffeinated and decaffeinated coffees. A significant increase ($p < 0.01$) in blood glucose was noted between the −60- and −30-minute means following nondecaffeinated, but not following decaffeinated coffee. The 0-minute means showed no significant difference between nondecaffeinated

Table 2.—Plasma Glucose Values (mg./100 cc.) Following Ingestion of Decaffeinated Coffee and Glucose Infusion

Subject	−60	−30	0	10	20	30	40	50	60 min.
1	100	83	88	260	233	220	198	173	150
2	100	—	—	295	250	215	198	163	140
3	75	75	75	285	251	208	175	158	145
4	85	88	80	190	185	165	160	115	95
5	85	93	85	260	220	200	—	143	125
6	80	81	83	195	153	125	105	93	80
7	63	63	70	230	198	160	128	113	105
8	70	75	75	260	235	203	153	125	113
Mean	82.3	79.7	79.4	246.9	215.6	187.0	159.6	135.4	119.1
(SE)	(4.7)	(3.7)	(2.4)	(13.7)	(12.2)	(11.8)	(13.1)	(10.0)	(8.9)

Table 3.—K Values (min.$^{-1}$) Observed Under Conditions of Decaffeinated and Nondecaffeinated Coffee Ingestion

Subject	Decaffeinated	Nondecaffeinated
1	.0107	.0112/min.
2	.0145	.0101
3	.0141	.0130
4	.0141	.0164
5	.0147	.0109
6	.0175	.0103
7	.0166	.0104
8	.0181	.0123
Mean (SE)	.0150 (.0008)	.0118 (.0007)/min.

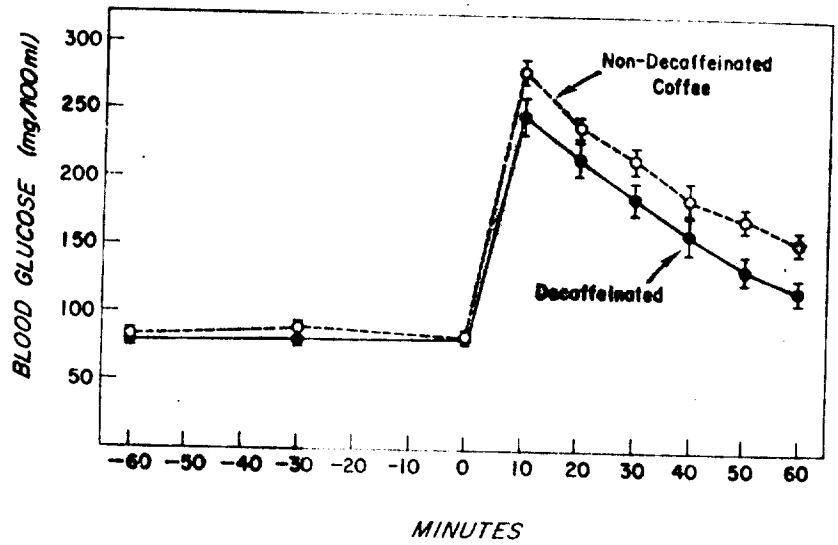

Fig. 1.—Blood glucose levels (mg./100 cc.) following ingestion of decaffeinated and nondecaffeinated coffees (−60 min.), and glucose infusion (0 min.) in eight humans. Brackets equal one SEM.

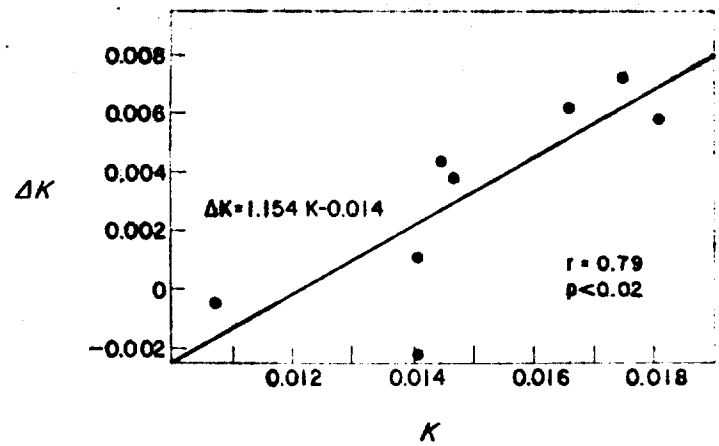

Fig. 2.—Scatter diagram illustrating relationship of difference between K values incurred under conditions of decaffeinated and nondecaffeinated coffees to control (decaffeinated) K value in eight subjects. Symbolic description of relationship is least-squares-best-fit; r, correlation coefficient; and p, significance of correlation.

Table 4.—Plasma Immunoreactive Insulin Values (μU/cc.) Following Ingestion of Nondecaffeinated Coffee and Glucose Infusion

Subject	−60	−30	0	10	20	30	40	50	60 min.
1	10	11	11	24	26	26	28	47	22
2	30	34	35	—	12	10	4	18	26
3	63	40	42	122	135	124	111	115	109
4	8	10	12	39	31	36	27	30	29
5	20	12	12	28	39	29	—	61	12
6	5	4	5	16	24	18	19	22	18
7	12	8	5	32	36	44	39	45	34
8	45	29	9	96	31	40	63	47	25
Mean	24.1	18.5	16.4	51.0	41.8	40.9	41.6	48.1	34.4
(SE)	(7.3)	(4.8)	(5.0)	(15.5)	(13.6)	(12.5)	(13.5)	(10.8)	(10.8)

coffee and decaffeinated coffee, but if the probably erroneous 0-minute level of glucose for nondecaffeinated coffee of subject 5 is omitted, the difference is significant ($p < 0.01$). Following glucose infusion, with the exception of the 40-minute mean, all blood glucose means were significantly higher ($p < 0.02$ to $p < 0.01$) following the ingestion of the nondecaffeinated coffee than the corresponding means following the ingestion of the decaffeinated coffee.

With two exceptions (subjects 1 and 4) the subjects manifested a lower K value, or prolonged fractional disappearance rate of glucose, following the ingestion of non-decaffeinated coffee compared to values obtained following the ingestion of decaffeinated coffee (Table 3). The means of the K values were statistically significantly different ($p < 0.05$). A significant inverse correlation ($r = -0.77$, $p < 0.02$), was noted between the control (decaffeinated) K value versus the degree of prolongation of half life of glucose induced by nondecaffeinated coffee. This is illustrated in Fig. 2.

Table 5.—Plasma Immunoreactive Insulin Values (μU/cc.) Following Ingestion of Decaffeinated Coffee and Glucose Infusion

Subject	−60	−30	0	10	20	30	40	50	60 min.
1	8	2	6	37	36	33	30	29	28
2	30	—	—	46	37	32	37	31	36
3	35	35	37	145	162	180	127	120	122
4	14	11	13	17	19	18	24	22	21
5	19	11	12	31	49	130	—	100	58
6	10	13	15	25	24	23	23	18	19
7	12	14	11	36	61	66	66	46	49
8	21	19	27	72	51	32	36	40	37
Mean	18.6	15.0	17.3	51.1	54.9	64.3	49.0	50.8	46.3
(SE)	(3.4)	(3.8)	(4.1)	(14.6)	(16.1)	(21.0)	(14.1)	(13.4)	(11.8)

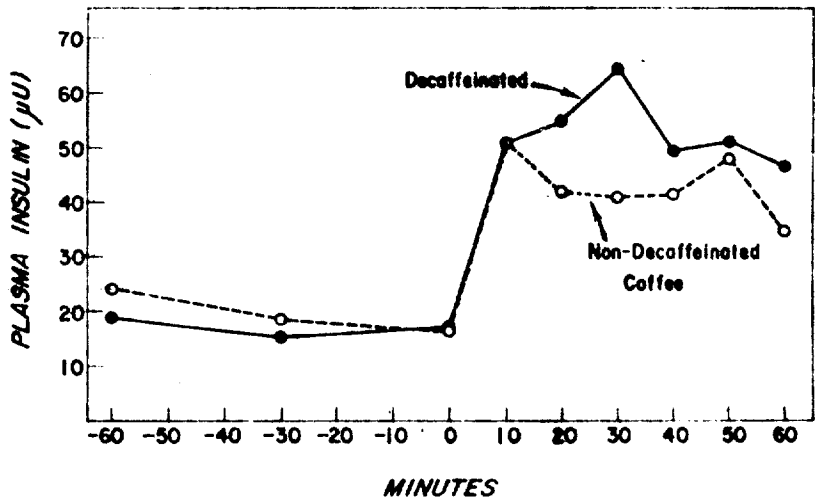

Fig. 3.—Plasma insulin levels (μU/cc.) following ingestion of decaffeinated and nondecaffeinated coffees (–60 min.), and glucose infusion (0 min.) in eight subjects.

Plasma Insulin Levels (Tables 4 and 5, Fig. 3)

No significant change was noted in plasma insulin means following the ingestion of either decaffeinated or nondecaffeinated coffees from −60 to 0 minutes, nor were significant differences detected between plasma insulin means under the two conditions. There was, however, an highly significant correlation between the means of the −60-, −30-, and 0-minute values for each individual on the two test occasions ($r = +0.94$, $p < .001$). This correlation implies acceptable quality control of the insulin determinations. Following glucose infusion the mean plasma insulin levels were lower at each corresponding point in time following nondecaffeinated than following decaffeinated coffee. Because of the variation of plasma insulin levels within individuals, none of these pairs of means, with the exception of the 20-minute sample ($p < 0.05$), were significantly different.

Insulin-to-glucose Ratios (Table 6)

To assess an effect of nondecaffeinated coffee upon the sensitivity of insulin

Table 6.—Insulin-to-glucose Ratio Under Conditions of Decaffeinated and Nondecaffeinated Coffee (μU/mg.)

Subject	Decaffeinated	Nondecaffeinated
1	.157	.118 μU/mg.
2	.174	.065
3	.699	.566
4	.133	.188
5	.388	.147
6	.176	.104
7	.347	.185
8	.246	.253
Mean (SE)	.290 (.067)	.203 (.056)

secretion to blood glucose,[14,15] the mean insulin response was divided by the mean blood glucose response following glucose infusion for the two conditions for each individual. With one exception (subject 4) the subjects had smaller insulin to glucose ratios following nondecaffeinated than following decaffeinated coffee. The means were statistically significantly different ($p < 0.05$).

Caffeine Content of Coffees

The nondecaffeinated coffee was found to contain 44.9 mg., the decaffeinated 6.5 mg., of caffeine per gram of the dry powdered material.

Discussion

The elevation of blood glucose, both in the fasting state and following a glucose load, appears to be both consistent and significant after the ingestion of nondecaffeinated coffee. These data support the earlier work of Jankelson et al.[2] in diabetic subjects, but contrast with the later work of Bellet and his colleagues.[5-7] It should be noted that the use of decaffeinated coffee as the control material offers a different, and perhaps better, control than the hot water used by Feinberg et al. In the study of Feinberg et al. the lowered and flatter oral glucose tolerance curves noted in his subjects given nondecaffeinated coffee might be accounted for by an effect upon gastrointestinal motility of nondecaffeinated coffee. The present study utilizing decaffeinated coffee as a control and intravenously administered glucose obviated this possibility. These workers administered the coffee and glucose simultaneously in contrast to the presently investigated one hour time lag.

The impairment in insulin secretion accompanying diabetes mellitus has been defined as ". . . a smaller than normal increment in insulin output in response to a given insulinogenic stimulus by glucose."[15] The present finding of a significant difference in insulin to glucose ratios induced by nondecaffeinated coffee compared to decaffeinated coffee is compatible with this definition. The mean plasma insulin levels were lower at each point in time following glucose infusion for nondecaffeinated compared to decaffeinated coffee and a significant difference existed at 20 minutes. Instead of, or in addition to, a direct effect of these constituents upon insulin it is conceivable that insulin secretion stimulators, par-

ticularly the intestinal polypeptide insulin releasing factors, may be inhibited by the constituents of nondecaffeinated coffee.

We are unable to adequately explain the divergence between our findings in humans and those of Bellet and his co-workers.[8] The findings by these investigators of an elevation of plasma free fatty acids, and their confirmation of the well known effect of increased catecholamine release following caffeine administration would seem to support the concept of a diabetogenic effect of caffeine on glucose metabolism.[5-7]

What constituent of nondecaffeinated coffee produces the observed changes? Coffee is a complex natural product and its decaffeination is relatively nonspecific, removing caffeine, other methyl xanthines, and small amounts of other compounds, as well. Herein may lie the explanation for the failure of previous studies[4] using pure caffeine, or different controls, to show the presently reported changes. In this context it should be emphasized that when the subjects were given 10 Gm. of decaffeinated coffee they were actually receiving approximately 64.5 mg. of caffeine.

The significant correlation between decaffeinated K values and the degree of change in K values following nondecaffeinated coffee, and the significant differences between the insulin to glucose ratios, suggest that the greater the sensitivity of insulin secretion to blood glucose, the greater the sensitivity to the effect of nondecaffeinated coffee upon the fate of a glucose load.

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REFERENCES

1. Cheraskin, E., Ringsdorf, W. M., Jr., Setyaadmadja, A. S. T. H., and Barre, R. A.: Effects of caffeine vs. placebo supplementation on blood glucose concentration. Lancet 1:1299, 1967.

2. Jankelson, O. M., Beaser, S. B., Howard, F. M., and Mayer, J.: Effect of coffee on glucose tolerance and circulating insulin in men with maturity-onset diabetes mellitus. Lancet 1:527, 1967.

3. Feinberg, I. J., Sandberg, H., DeCastro, O., and Bellet, S.: The effects of coffee ingestion on oral glucose tolerance curves in normal human subjects. Metabolism 17:916, 1968.

4. DeCastro, O. A. P., Sandberg, H., Feinberg, L. J., and Bellet, S.: Effects of various routes of administration on oral and intravenous glucose tolerance tests in dogs. Metabolism 18:163, 1969.

5. Bellet, S., Kershbaum, A., and Aspe, J.: The effect of caffeine on free fatty acids. A preliminary report. Arch. Int. Med. 116: 750, 1965.

6. —, Feinberg, L. J., Sandberg, H., and Hira Bauashi, M.: The effects of caffeine on free fatty acids and blood coagulation parameters of dogs. J. Pharmacol. Exp. Ther. 159:250, 1968.

7. —, Roman, L., DeCastro, O., Kim, K. E., and Kershbaum, A.: Effect of coffee ingestion on catecholamine release. Metabolism 18:288, 1969.

8. Butcher, R. W., and Sutherland, E. W.: Adenosine 3′, 5′-phosphate in biological materials. J. Biol. Chem. 237:1244, 1962.

9. Handler, J. S., Butcher, R. W., Sutherland, E. W., and Orloff, J.: The effect of vasopressin and of theophylline on the con-

centration of adenosine 3′, 5′-phosphate in the urinary bladder of the toad. J. Biol. Chem. 240:4524, 1965.

10. Axelrod, J., and Reichenthal, J.: The fate of caffeine in man and a method for its estimation in biological material. J. Pharmacol. Exp. Ther. 107:519, 1953.

11. Soeldner, J. S., and Slone, D.: Critical variables in the radioimmunoassay of serum insulin using the double antibody technique. Diabetes 14:771, 1965.

12. Snedecor, G. W.: Statistical Methods (ed. 5). Ames, Iowa, Iowa State University Press, 1956, p. 447.

13. Bruning, J. L., and Kintz, B. L.: Computational handbook of statistics. Glenview, Ill., Scott, Foresman, 1968, pp. 12, 152.

14. Perley, M., and Kipnis, D. M.: Plasma insulin response to glucose and tolbutamide of normal weight and obese diabetic and non-diabetic subjects. Diabetes 15:867, 1966.

15. Seltzer, H. S., Allen, E. W., Herron, A. L., and Brennan, M. T.: Insulin secretion in response to glycemic stimulus: relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus. J. Clin. Invest. 46:323, 1967.

16. Davidoff, F. F.: Oral hypoglycemic agents and the mechanism of diabetes mellitus. New Eng. J. Med. 278:148, 1968.

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Am. J. Cardial. 19:339-343. 1967.

Myocardial Infarction in Young Men*

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CORONARY atherosclerosis is the leading single cause of death in the United States both among the elderly and in those below age 65.[1] It is also the leading cause of death from disease among active-duty personnel of the United States Army. As a cause of death and as a cause of separation from the service for physical disability, it exceeds all forms of malignancy combined. The average age of separation for disability from this disease in the Army is 44 years; the median age of death among those dying on active duty is 43 years.[2]

Study of coronary atherosclerosis as it manifests itself precociously in younger individuals, before degenerative and aging processes have developed, should be most fruitful in defining clearly etiologic and risk factors of this epidemic disease. The vital factors to determine are those which predispose young male adults to myocardial infarction, since the disease then strikes down individuals in the prime of their productive lives. The majority of patients who die of coronary atherosclerosis die before they reach a physician. In the military service 60 per cent of all coronary deaths occur suddenly before individuals are able to reach medical facilities.[2] The same is true in civilian life. In the Framingham study 77 per cent of the patients with initial infarcts, who died within two weeks of the attack, succumbed before reaching a hospital.[3] These facts clearly indicate that the only successful approach to this medical problem must be a *preventive* one.

This study was designed to identify further risk factors associated with the development of myocardial infarction in young male adults. More such studies are needed not only to establish firmly the major risk factors of coronary atherosclerosis but also to eliminate those factors which have been proposed on the basis of inadequate or inconclusive evidence. This can be accomplished by pooling data from studies such as ours.

MATERIAL AND METHODS

One hundred male military personnel who survived a transmural myocardial infarction at age 40 or less were studied at Walter Reed General Hospital during the period 1960 to 1964 inclusive. An effort was made to obtain age-matched, military control patients from the same hospital who were hospitalized for reasons other than cardiovascular or other chronic or wasting diseases. Patients with acute orthopedic injuries or fractures were chosen, since they afforded histories of acute, sudden onset of their illness; this was the focal point of an extensive questionnaire designed to clarify medical histories, personal habits and physical and mental status immediately prior to the event which led to hospitalization.

Twenty parameters were evaluated: serum cholesterol, total serum lipids, arterial blood pressure, height, weight, cigarette smoking, relative consumption of saturated and unsaturated fats, emotional tension, race, coffee and alcohol consumption, degree of physical activity, history of peptic ulcer disease, hematocrit, frequency and extent of baldness, and family history of coronary disease, diabetes mellitus or gout. Both groups of patients completed identical questionnaires. Near the end of the study it became evident that there were insufficient orthopedic patients of appropriate age; therefore, a few age-matched patients, hospitalized for other than chronic diseases, were included as controls. When both investigators left Walter Reed General Hospital, only 72 age-matched controls had been studied. However, numerous striking, statistically significant differences between the two groups were evident and form the basis of this report.

Statistical Methods: The F test, derived from analysis of variance, was used to test for significant differences of the measurement data. Enumeration data were analyzed for significance by the chi square test; when the numbers were small, the corrected chi square test according to Yates was applied.[4]

RESULTS

Table 1 summarizes the differences between the two groups. A statistically significant difference was demonstrated in 12 parameters;

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TABLE I
Parameters Evaluated in 100 Patients Who Survived a Myocardial Infarction at Age 40 or Less and in 72 Noncardiac Controls (Mean ± Standard Error)

	Infarcts	Controls	p
Age (yr.)	35 ± 0.4	34 ± 0.5	N.S.
Height (in.)	69.3 ± 0.25	70.6 ± 0.33	<0.01
Overweight (lb.)*	32.35 ± 2.55	14.94 ± 2.44	<0.001
Serum cholesterol (mg./100 ml.)	315.68 ± 7.97	230.44 ± 4.74	<0.001
Serum lipids, total (mg./100 ml.)	982 ± 34.18	793 ± 19.97	<0.001
Family history of coronary artery disease	47%	20%	<0.001
History of hypertension	21%	1%	<0.01
Observed hypertension	28%	10%	<0.02
Smoked > 1 pack cigarettes/day	60%	42%	<0.05
Estimated consumed > av. sat. fat	25%	11%	<0.05
Estimated consumed < av. unsat. fat	40%	15%	<0.01
Increased tension at time of event	52%	28%	<0.01
Race: Negro	7%	24%	<0.01
Physical activity < av.	25%	20%	N.S.
> Av. baldness	24%	13%	N.S.
Consumed > 4 cups coffee/day	59%	51%	N.S.
Consumed > 2 oz. whiskey/day	29%	23%	N.S.
History of peptic ulcer	7%	8%	N.S.
History of gout	2%	1%	N.S.
Family history of diabetes mellitus	14%	17%	N.S.
Hematocrit	45.1 ± 1.8	45.3 ± 1.1	N.S.

* Overweight according to Metropolitan Life Insurance Co. tables.[9]
Av. = average; Sat. = saturated; N.S. = not significant.

some of these interrelate. The four most striking factors in the infarct group were elevated serum cholesterol and total lipids, family history of coronary artery disease and obesity (Fig. 1 and 2). Not only were patients who sustained myocardial infarctions more obese, but they had also gained more weight while in the military service than those in the control group. Less striking but statistically significant factors associated with myocardial infarction were hypertension, short stature, cigarette smoking, consumption of saturated fats, low intake of unsaturated fats, emotional tension and Caucasian race. On the other hand, no significant difference was found in coffee consumption, alcohol consumption, degree of physical activity, history of peptic ulcer, hematocrit, frequency and extent of baldness, or family history of diabetes mellitus or gout.

DISCUSSION

Serum Lipids: Numerous studies have indicated elevated serum cholesterol as an extremely important risk factor in the genesis of coronary atherosclerosis in young individuals.[5,6] Our study fully confirms this association and suggests that total serum lipid correlate almost as well with early atherosclerosis. The fact that the correlation with elevated serum cholesterol is not so striking in older-age groups[7,8] in no way disproves the importance of cholesterol in the genesis of atherosclerosis but merely suggests that other factors also become operative in older individuals in whom atherosclerosis is already established.

Obesity: Some authors have questioned the importance of obesity as a factor in atherosclerosis. It was one of the most striking factors in this study. Sixty-five per cent of the patients in the infarct group were obese versus 38 per cent of the controls (20 per cent or more above standard weight)[9]; only 5 per cent of the infarct group were below standard weight, while 24 per cent of the controls were underweight. The infarct group had gained an average of 29 pounds since entering the military service, while the control group had gained an average of 14 pounds; only 6 per cent of the infarct group had lost weight compared to 21 per cent of the control group. Experimental data suggest that weight gain may elevate serum lipid levels in man[10]; such weight gain may have contributed to the high lipid levels in the infarct group.

Family History of Coronary Disease: The increased family history of coronary artery disease undoubtedly reflects predisposing enzymatic

genetic factors; however, this may not be as significant as it appears, since family patterns of eating and of obesity may also be involved. In addition, survivors of a myocardial infarction are more likely to recall distant relatives with heart attacks or are reminded of such by those who write or visit them during their convalescence; orthopedic patients would be less likely to be reminded of a family history of coronary artery disease. The failure to find an increased family history of diabetes mellitus among the patients with infarction was unexpected, and is unexplained. Patients with known diabetes are not inducted into the military service, and none of the patients in either group had overt diabetes.

Short Stature: Garn et al.[11] have emphasized the greater incidence of myocardial infarction among individuals of short stature. We found a smaller but still significant difference. Their coronary group averaged 1.8 in. shorter than the controls and ours, 1.3 in.

Hypertension: The Framingham and life insurance studies have emphasized the importance of hypertension as a significant risk factor in coronary artery disease.[8,9,12] Our study supports this concept. Twenty-one per cent of the infarct group had been told they had elevated

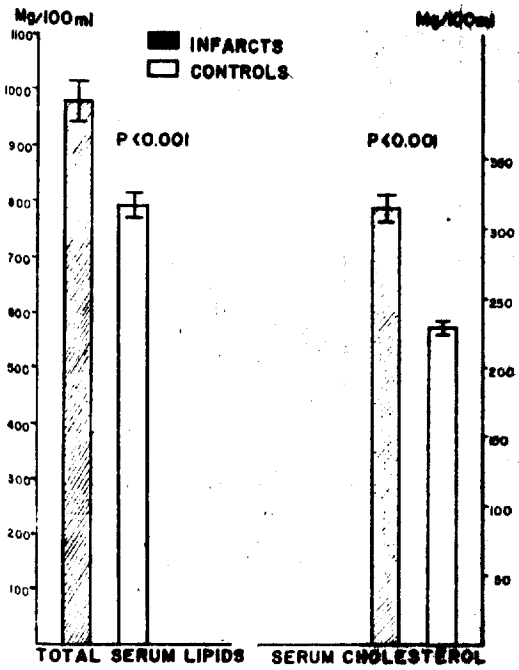

Fig. 1. Total serum lipid and cholesterol levels (mean ± S.E.) in infarction and control groups.

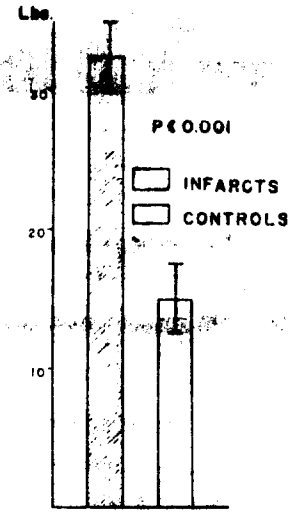

Fig. 2. Obesity (expressed as pounds overweight (mean ± S.E.)) in infarct and control groups.

blood pressure prior to the event which led to their hospitalization, while only 1 per cent of the control group had knowledge of prior hypertension. Although myocardial infarction often lowers elevated blood pressure, the infarct group at the time of evaluation had approximately three times the incidence of observed hypertension as the controls.

Smoking: This study also confirmed a relation between cigarette smoking and coronary atherosclerosis. There was not only a higher percentage of heavy smokers among the infarct group (60% vs. 42%) but also a lower incidence of noncigarette smokers (13% vs. 30%).

Saturated and Unsaturated Dietary Fat: While many studies have confirmed the relation between elevated serum cholesterol and coronary artery disease, many have failed to demonstrate a difference between the amount and type of fat and cholesterol ingested.[13] Both of our groups were given a list of common foods containing large amounts of saturated fats, and another list of foods containing largely unsaturated fatty acids. The coronary group not only indicated that they tended to consume more than average amounts of foods high in saturated fats but demonstrated an even more significant aversion to foods high in polyunsaturated fats than did the control group.

Emotional Tension: Several investigators have claimed that emotional tension is an important factor in causing coronary artery disease.[14,15] Others have been skeptical because of the difficulties inherent in defining and quantitating

TABLE II
Age Distribution of 100 Male Patients with Myocardial Infarction

No. Patients	Ages		No. Patients	Ages	
13	40		1	30	
15	39		3	29	
5	38		0	28	
11	37		0	27	
7	36	90%	1	26	10%
9	35		2	25	
12	34		1	24	
7	33		2	23	
6	32				
5	31				

nervous tension. For what it is worth, approximately twice as many of the coronary group considered themselves under heavy emotional tension prior to the event that led to their hospitalization than did the orthopedic group.

Baldness: While there was a somewhat higher incidence of baldness in the coronary group, the difference was not statistically significant. These findings are not surprising since studies indicate that although testosterone is an essential inciting agent of common baldness in men, the level of this hormone does not correlate with the incidence of baldness.[16]

Race: The difference in the number of Negro soldiers in the two groups is quite striking and surely suggests that young Negro men are less prone to develop myocardial infarction than are Caucasian men of the same age. The 7 per cent of Negroes in the infarct group is distinctly below the percentage of Negro soldiers in the Army. However, the 24 per cent of Negroes in the control group exceeds the expected incidence based on Army population distribution. These findings correlate with reports of the Metropolitan Life Insurance Company that male adult Negro Americans have a higher death rate from accidents but a lower death rate from coronary heart disease than male Caucasians.[17,18]

Physical Activity: There has been recent emphasis on the risks of a sedentary existence and the value of physical activity in preventing coronary atherosclerosis. The Framingham study indicated that patients with coronary artery disease had a low vital capacity. We did not measure vital capacity, but our patients completed questionnaires about their accustomed degree of physical activity. No significant difference between the two groups was evident.

Hematocrit: Our study likewise failed to confirm the contention of Burch and DePasquale[19] and Mayer[20] that coronary patients have elevated hematocrits. The mean hematocrit in our infarct group was 45.1, while the mean control value was 45.3. The average age of Burch and DePasquale's coronary patients was 24 years, and Mayer's patients 17 years older than ours; hence, it might be argued that elevation of the hematocrit predisposes to the complications of atherosclerosis in the elderly. Our data provide no evidence that the hematocrit level is a significant factor in causing myocardial infarction in young men.

Premonitory Pain: Forty-five per cent of the infarct group gave a history of premonitory chest pain prior to myocardial infarction, while 15 per cent of the controls who were not considered to have evidence of clinical coronary artery disease gave a history of prior chest pain. This 30 per cent difference between the two groups would suggest that chest pain is a fairly frequent, early manifestation of clinical coronary atherosclerosis that precedes myocardial infarction in young men. Presumably, about a third of the chest pains observed are unrelated to coronary atherosclerosis.

Age: Only about 5 per cent of myocardial infarctions occur at age 40 or less.[19] Table II indicates the age distribution of this selected group of young coronary patients and shows that, while the clinical disease may be manifested in early adult life, there is a striking increase in the incidence of the disease with aging in this young group of patients. Only 10 per cent of our hundred patients sustained their myocardial infarctions at age 30 or below, and half were 36 years or older at the time of their initial infarction.

SUMMARY

One hundred military men who survived a transmural myocardial infarction at age 40 or below were compared with age-matched military controls. Twenty possible etiologic factors were analyzed by questionnaire, physical examination and laboratory studies. A statistically significant difference was found in 12 parameters. The most striking correlation found in the infarct group was the elevated serum cholesterol, elevated total serum lipids, obesity and a family history of coronary artery disease. Other factors associated to a statistically significant degree with myocardial infarction were hypertension, short stature, ciga-

rette smoking, consumption of saturated fats, low intake of unsaturated fats, emotional tension, Caucasian race and family history of hypertension.

No association was found with coffee consumption, alcohol consumption, degree of physical activity, history of peptic ulcer, hematocrit, frequency and extent of baldness, or family history of diabetes mellitus or gout.

Pooling of data from several studies such as this is urgently needed to firmly establish the major risk factors of coronary atherosclerosis and to eliminate many factors that have been proposed on the basis of inadequate or inconclusive evidence.

References

1. Fact Sheet: Atherosclerosis. National Health Education Committee, Inc. New York, 1964.
2. Walker, W. J. and Sherman, J. L. Atherosclerosis in the Armed Forces: Should the military diet be altered. *Mil. Med.*, 127: 205, 1962.
3. Kannel, W. B., Dawber, R. R., Kagan, A., Revorske, N. and Stokes, J. Factors of the risk in the development of coronary heart disease: Six year follow-up experience. *Ann. Int. Med.*, 55: 1, 1961.
4. Batson, H. C. An Introduction to Statistics in the Medical Sciences. Minneapolis, 1963. Burgess Publishing Co.
5. Kagan, A., Dawber, T. R., Kannel, W. B. and Revotski, N. The Framingham study: A prospective study of coronary heart disease. *Fed. Proc.*, 21 (No. 4, part 2): 52, 1962.
6. Gertler, M. M., Garn, S. M. and White, P. D. Diet, serum cholesterol and coronary artery disease. *Circulation*, 2: 696, 1950.
7. Gofman, J. W. Atherosclerosis 1965. Lyman G. Dull Memorial Lecture, Council on Atherosclerosis, American Heart Association, Miami, Oct. 13, 1965.
8. Paterson, J. C., Armstrong, R. and Armstrong, L. C. Serum lipid levels and the severity of coronary and cerebral atherosclerosis in adequately nourished men 60 to 69 years of age. *Circulation*, 27: 229, 1963.
9. Desirable weights for men and women. *Statist. Bull. Metrop. Life Insur. Co.*, 40: 3, 1959.
10. Walker, W. J. et al. Effect of weight reduction and caloric balance on serum lipoprotein and cholesterol levels. *Am. J. Med.*, 14: 654, 1953.
11. Garn, S. M., Gertler, M. M., Levine, S. A. and White, P. D. Body weight versus weight standards in coronary artery disease and a healthy group. *Ann. Int. Med.*, 34: 1416, 1951.
12. Body Build and Blood Pressure Study. Society of Actuaries, Chicago, 1959.
13. Keys, A. Epidemiological aspects of atherosclerosis and the diet. *Geriatrics*, 19: 542, 1964.
14. Russek, H. I. Emotional stress and coronary heart disease in American physicians. *Am. J. M. Sc.*, 240: 711, 1960.
15. Friedman, M. and Rosenman, R. H. Association of specific overt behavior pattern with blood and cardiovascular findings. *J.A.M.A.*, 169: 1286, 1959.
16. Hamilton, J. B. Patterned loss of hair in man: Types and incidence. *Ann. New York Acad. Sc.*, 53: 708, 1951.
17. Improved mortality among Colored policyholders. *Statist. Bull. Metrop. Life Insur. Co.*, 43: 6, 1962.
18. Types of fatal heart disease. *Statist. Bull. Metrop. Life Insur. Co.*, 42: 8, March, 1961.
19. Burch, G. E. and DePasquale, N. P. The hematocrit in patients with myocardial infarction. *J.A.M.A.*, 180: 63, 1962.
20. Mayer, G. A. Hematocrit and coronary heart disease. *Canad. M.A.J.*, 93: 1151, 1965.

Warren-'69
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Metabolism of xanthine alkaloids in man

Despite the world-wide occurrence of xanthine alkaloids (caffeine, theobromine and theophylline) and the consumption of relatively enormous quantities in a variety of beverages, remarkably little is known about their metabolism in man.

Urinary excretion products have been studied following the ingestion of a large dose of one particular xanthine. CORNISH AND CHRISTMAN[1] found methylated uric acids as well as 1-methyl-, 7-methyl-, and 1,7-dimethylxanthine (paraxanthine) after caffeine ingestion. SCHMIDT AND SCHOYERER[2] in similar studies detected caffeine, theobromine, paraxanthine and 1-methylxanthine in the urine of subjects treated with 300 mg caffeine orally.

It was decided to attempt the separation and identification of caffeine and its metabolites in human blood as a preliminary to further studies of caffeine metabolism in man. After many trials a two-stage system for thin-layer chromatographic separation was devised which achieved an adequate separation of the xanthines after a preliminary removal of blood lipids.

Plasma or red cells, separated from heparinized blood, was extracted twice by shaking with 3 volumes of chloroform (chloroform extracts methylxanthines but not uric acids). The chloroform extracts were clarified by centrifugation and evaporated to dryness. The extracts were then applied to plates prepared by slurrying Kieselgel G according to STAHL with phosphate buffer (pH 6.8) and spreading on glass plates to a thickness of 300 μ. The plates were used after drying and heating to 105° for 30 min. Plasma extracts (20 μl) were applied for runs of 15 cm and 2 μl of appropriate standards were applied at concentrations of 2 μg/ml in 2 N NH_3. Detection was effected by spraying with I_2/KI in ethanol (which coloured the plates brown all over) followed by 95% ethanol containing 25% conc. HCl. By this procedure caffeine was revealed as a brown spot and the dimethylxanthines as purple-blue spots against a yellow background. Spots faded rapidly and required immediate marking. The sensitivity was about 1.0 μg. Since lipids are also stained by this procedure the plates were also sprayed with 5% phosphomolybdic acid in ethanol and heated to 120° for 5 min. Lipids were revealed as blue spots.

Many solvent systems were tried for separation of caffeine and dimethylxanthines. Chloroform–ethanol (9:1) gave no separation of theobromine and paraxanthine and lipids streaked considerably over the xanthine area. Altering the ratio of the two solvents gave no improvement. Butanol–acetic acid–water (4:1:1) was much more successful. Although runs were lengthened to 3 h the separation was greatly improved. R_F values were: caffeine 0.42; theobromine 0.38; paraxanthine 0.47. Butanol–formic acid–water (33:1:7) gave very similar results.

The presence of blood lipids in the chloroform extracts at first complicated the separation of the xanthines since the lipids ran into the same areas in these solvent systems. However, the problem was resolved by the discovery that when the extracts were first run in petroleum ether (b.p. 60–80°) the lipids moved a long way (R_F approx. 0.70) while the xanthines remained on the start line. A general procedure was therefore adopted of pre-running the extracts in petroleum ether and then re-running in butanol–acetic acid–water.

This procedure has now been applied to a series of human volunteers with the following results. (1) Habitual consumers of beverages containing caffeine require about 7 days of abstention from them before caffeine completely disappears from their blood extracts. (2) In such "decaffeinated" subjects administration of 500 mg caffeine orally leads to the appearance of caffeine and paraxanthine in both red cells and plasma within 3 h. (3) Caffeine and paraxanthine then disappear from the blood extracts within 24 h.

From these results it would appear that the first step in the metabolism of caffeine in man is the removal of the 3-methyl group and the formation of 1,7-dimethylxanthine (paraxanthine). Why it takes so long to "decaffeinate" the habitual caffeine consumer as compared with the "decaffeinated" subject treated with an acute dose of caffeine is not known and further work is being undertaken.

I should like to express my gratitude for the enthusiastic collaboration of all the B.Sc. students who have taken part in this work over the past two years.

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1 H. H. CORNISH AND A. A. CHRISTMAN, *J. Biol. Chem.*, 228 (1957) 315.
2 G. SCHMIDT AND R. SCHOYERER, *Deut. Z. Ges. Gerichtl. Med.*, 57 (1966) 402.

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THE METABOLISM OF CAFFEINE AND THEOPHYLLINE*

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Following the ingestion of caffeine and theophylline by man (1–5), the Dalmatian dog (6), and the rat[1] (7), an increased urinary excretion of substances which reduce alkaline phosphotungstate solutions was observed. Although earlier workers in this field had stated that the increased reduction was due to uric acid, Myers and Wardell (4) in 1928 suggested that the chromogens from the methylxanthines might be methyluric acids which resulted from the oxidation of the carbon in position 8. Since only those methyluric acids (1,3-dimethyluric acid, 1-methyluric acid, and 3-methyluric acid) which lack a methyl group in position 7 reduce the alkaline phosphotungstate reagents to a degree comparable to that of uric acid, demethylation of caffeine at position 7 must accompany its oxidation at position 8.

By the ordinary colorimetric methods, it was not possible to determine whether the increased excretion of chromogenic material obtained after the feeding of caffeine or theophylline was due to these reducing methyluric acids or to uric acid produced by complete demethylation and oxidation of the methylxanthines. By the use of the more specific uricase method, Buchanan, Christman, and Block (5, 8) showed that in man there was no significant increase in the excretion of uric acid following the ingestion of caffeine or theophylline. These workers also observed that, on the addition of the three chromogenic methyluric acids to urine, only 1-methyluric acid could be precipitated along with uric acid by the ammoniacal silver solution of Benedict and Hitchcock (9). Since in the urines, collected after the ingestion of caffeine and theophylline, a considerable portion of the non-uric acid chromogens was precipitable by ammoniacal silver, the presence of 1-methyluric acid was strongly indicated. More recently Brodie *et al.* (10) isolated 1,3-dimethyluric acid from the urine of human subjects who had ingested theophylline.

Christman and Seamens[1] have also observed that subsequent to the oral administration of caffeine and theophylline to rats, the largest portion of

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[1] Christman, A. A., and Seamens, M., unpublished data.

the chromogenic material in the urine was not uric acid. Evidence was again obtained that, after caffeine feeding, the greater part of the urinary non-uric acid chromogen was 1-methyluric acid. A further study by the methods of paper chromatography to determine the nature of the non-uric acid chromogens, found in the urine of man, the rat, and the rabbit following the ingestion of caffeine and theophylline, is reported in the present paper. A preliminary report of some of these data was presented in 1951 (11).

EXPERIMENTAL

Chromatographic Technique—Urines, collected for 24 hour periods, were preserved with acid or toluene. The control urines were collected during the 24 hour period immediately preceding the administration of the methylated xanthines. Common sources of methylated xanthines were removed from the diet for several days preceding and during the collection of urines. For the chromatographic study, aliquots of the control urines were supplemented individually with uric acid, 1-methyl-, 3-methyl-, or 1,3-dimethyluric acid in 0.1 per cent lithium carbonate solution. In any one chromatographic analysis, equal aliquots of unsupplemented control urine, supplemented control urine, and urine obtained after administration of caffeine or theophylline were chromatographed simultaneously on sheets or strips of Whatman No.1 filter paper. Sheet chromatograms were obtained in phenol saturated with water (Solvent A) by descending capillarity (12), strip chromatograms were obtained by ascending capillarity (13) in a single phase mixture of *n*-butanol, distilled water, benzene, and methanol, 1:1:1:2 by volume (Solvent B), and a single phase mixture of *n*-butanol, distilled water, and glacial acetic acid, 5:5:3 by volume (Solvent C). For suitable development, chromatography in Solvent A required 30 hours; in Solvent B, 16 to 18 hours; and in Solvent C, 30 to 35 hours. After the chromatograms were allowed to dry in air, the positions of the solvent fronts were located by a faint coloration on the paper when Solvent A was used and by the fluorescence in ultraviolet light of impurities which accompanied the advancing front of liquid when Solvents B and C were used. The paper was sprayed first with a urea-cyanide carbonate reagent (14), followed by an arsenophosphotungstic acid solution (15). Areas which were colored blue denoted the presence of chromogens. After drying in air these areas were outlined in pencil, since the color gradually faded. The R_F values were computed from the point of farthest advance of the chromogen in relation to that of the solvent front.

Urine Analysis—Total chromogens, non-uric acid chromogens, and uric acid were determined by the method of Buchanan and coworkers (8). "Total chromogens" and "non-uric acid chromogens," expressed in terms

of uric acid, are identical with "total color" and "residual color" as used by these workers. The Benedict-Hitchcock indirect method for uric acid, as modified by Buchanan *et al.* (8), was used in some cases. 24 hour excretions of creatinine were determined as a check on the adequacy of the urine collections.

Results

Identification of Methyluric Acids in Urine of Human Subject Following Ingestion of Caffeine—A 24 hour control urine from a 75 kilo male subject was collected after several days on a diet essentially free of methylated xanthines. 1 gm. of caffeine in divided doses was taken over a 5 hour period, and the 24 hour experimental sample was collected. Both urines were diluted to a volume of 2 liters. The creatinine excretion for the 24 hour control and experimental periods was 1.95 and 1.93 gm. respectively. The excretion of true uric acid for these periods was 681 and 654 mg. respectively. The non-uric acid chromogens in terms of uric acid were increased by 251 mg. (45 to 296 mg.) after caffeine ingestion. Uric acid, as determined by the modified Benedict-Hitchcock method, rose from a control level of 664 to 812 mg. This indicates that approximately 60 per cent of the increase in non-uric acid chromogens following the ingestion of caffeine was precipitable with ammoniacal silver. This result and the chromatographic data recorded in Table I support the conclusion that 1-methyluric acid is an important metabolite of caffeine in the human body. Table I also shows that some 1,3-dimethyluric acid may be a product of caffeine metabolism in man, but there is no evidence for the presence of 3-methyluric acid in the experimental urine.

Identification of Methyluric Acids in Urine of Human Subject Following Ingestion of Theophylline—After a preliminary period of 4 days on a diet free of methylxanthines, the same subject used in previous experiments ingested 0.7 gm. of theophylline in divided doses over a period of several hours. Creatinine determinations on the 24 hour samples of the control and experimental urines indicated a satisfactory collection of urine. Total chromogens increased from a level of 576 mg. in the control urine to 818 mg. in the urine collected after the ingestion of theophylline. On the basis of earlier work in this laboratory (5) it can be assumed that this increase in chromogens (242 mg.) was not due to uric acid. The results of the chromatographic study of this urine are found in Table II. After the ingestion of theophylline, the urine contained a chromogen which migrated at a rate similar to that of 1,3-dimethyluric acid in three different solvents. In Solvents B and C, 1-methyluric acid added to the control urines gave distinct spots, with R_F values of 0.57 and 0.78 respectively. Similar spots with R_F values of 0.55 and 0.71 were found in the urine after theophylline.

3-Methyluric acid added to the control urine did not separate cleanly from a normal chromogen in the control urine which had R_F values of 0.34 to 0.35 and 0.61 to 0.64 in Solvents B and C respectively. In the urine collected after theophylline ingestion there was no indication that 3-methyluric acid was present. It is concluded, therefore, that 1,3-dimethyluric acid and 1-methyluric acid are the principal chromogenic substances excreted in the urine of man after ingestion of theophylline.

TABLE I

R_F Values of Chromogenic Methyluric Acids Added to Control Urine and of Chromogens Present in Urine of Human Subject before and after Ingestion of 1 Gm. of Caffeine

The volumes of the control and experimental urines were 2 liters. The aliquots of urine chromatographed were 200 μl. in Solvent A and 100 μl. in Solvent C. The amount of each methyluric acid added to the control urine was 40 γ.

	Solvent A		Solvent C	
	Trial 1	Trial 2	Trial 1	Trial 2
Control urine*	0.30–0.31	0.32–0.33	0.45–0.51	0.55–0.59
" " + 1,3-dimethyluric acid	0.89, 0.89			
Control urine + 3-methyluric acid		0.71, 0.72	0.63†	0.71
Control urine + 1-methyluric acid	0.71, 0.71	0.67, 0.68	0.70	0.80
Urine after caffeine ingestion	0.72, 0.72, 0.88, 0.89 ‡	0.68, 0.68	0.72	0.61

* The principal chromogen found in the control urine which has an R_F of 0.30 to 0.33 in Solvent A and 0.45 to 0.59 in Solvent C is uric acid. Similar spots from this substance occur on all chromatograms, but have not been recorded.

† Did not separate cleanly from the compound with R_F 0.45 to 0.51.

‡ Faint spot.

Identification of Methyluric Acids in Urine of Rats Following Oral Administration of Theophylline—Six male albino rats, each weighing approximately 300 gm., were maintained on a synthetic diet for several weeks prior to the experiment. The urine excreted by all the rats during a 24 hour control period was pooled, diluted to 500 ml. with distilled water, and filtered. 20 mg. of caffeine in 2 ml. of water were administered by stomach tube to each of three animals of the group on 2 successive days and 20 mg. of theophylline in 2 ml. of water were administered to each of the remaining animals on the same days. The urines of each of the two groups collected over the 48 hour period were pooled, diluted to 500 ml., and filtered. The increase in total chromogens following the ingestion of 120 mg. of caffeine

was only 4.9 mg. over the control level, whereas the increase following the same amount of theophylline was 31.6 mg. Because the increase in total chromogens after the administration of caffeine was so small, chromatographic analysis of the urine was not attempted. The urine collected after theophylline was chromatographed in Solvents B and C and compared with the control urine to which 1-methyluric, 3-methyluric, and 1,3-dimethyluric acids, had been added. In Solvent B, 1,3-dimethyluric, 1-methyluric, and 3-methyluric acids added to the control urine gave R_F values of 0.86,

TABLE II

R_F Values of Chromogenic Methyluric Acids Added to Control Urine and of Chromogens Present in Urine of Human Subject before and after Ingestion of 0.7 Gm. of Theophylline

The volumes of the control and experimental urine were 1500 and 2065 ml. respectively. The aliquots of urine chromatographed were 200 μl. The amount of each methyluric acid added to the control urine was 40 γ.

	Solvent A	Solvent B	Solvent C
Control urine*	0.25–0.27	0.19–0.21 0.34–0.35†	0.51–0.52 0.61–0.64†
Control urine + 1,3-dimethyluric acid	0.88	0.69	0.86
" " + 1-methyluric acid	0.68, 0.69	0.57	0.78
" " + 3-methyluric "		0.49‡	0.66§
Urine after theophylline ingestion	0.85, 0.86, 0.64, 0.65	0.68 0.55	0.83 0.71

* The slow moving chromogen found in control urine is probably uric acid. The same spots occur in all of the chromatograms, but are not recorded.

† Very faint spots.

‡ Did not separate from spot in control urine of R_F 0.34 to 0.35.

§ Did not separate from spot in control urine of R_F 0.61 to 0.64.

0.70, and 0.58 respectively. The urine collected after theophylline feeding contained chromogens which had R_F values of 0.87 and 0.66. This was strong evidence for the presence of 1,3-dimethyluric acid. The chromogen with an R_F value of 0.66 could be due to either 1-methyluric acid or 3-methyluric acid or to a mixture of both. A further study with Solvent C, however, showed that 3-methyluric acid was not present in amounts that could be detected by chromatography. In the latter solvent, the R_F values of 1,3-dimethyluric acid, 1-methyluric acid, and 3-methyluric acid were 0.91, 0.80, and 0.58. Chromogens were present in the rat urine, after feeding theophylline, which had R_F values at 0.91 and 0.78, but no detectable chromogen with an R_F comparable to that of 3-methyluric acid. As a further check, aliquots of the urine collected after theophylline feeding

were supplemented separately with 1-methyluric acid and with 1,3-dimethyluric acid. The supplemented and unsupplemented aliquots were then chromatographed in Solvent A and the color developed in the usual manner. By the use of this solvent, one chromogen in the experimental urine with an R_F in the area between 0.50 and 0.60 and a second chromogen with an R_F in the area between 0.80 and 0.90 were found. The urine supplemented with 1,3-dimethyluric acid showed an enhancement of the color at the higher R_F value, whereas the urine supplemented with 1-methyluric acid showed enhancement of color in the region between 0.50 and 0.70. Consideration of all of the chromatographic data leads to the conclusion that the increase in chromogens in rat urine after theophylline ingestion is principally due to 1-methyluric acid and 1,3-dimethyluric acid. The metabolism of theophylline by man appears to be similar to that by the rat.

Identification of Methyluric Acids in Urine of Rabbit after Oral Administration of Theophylline and Caffeine—A male albino rabbit, 3 kilos in weight, was maintained on a diet of oats, cabbage, and tap water. On each of 3 consecutive days, following the collection of a 24 hour control urine, 200 mg. of theophylline dissolved in approximately 0.07 per cent sodium carbonate were administered by stomach tube. 24 hour samples of urine collected during the 3 days of theophylline administration and a 24 hour postfeeding sample of urine were collected. It was found in a preliminary experiment that the control urine contained a chromogen (presumably uric acid) with an R_F value of 0.28 to 0.32 in Solvent A. The 24 hour sample of urine, collected after the first administration of theophylline, contained a second chromogen which, under the same conditions, gave an R_F value of approximately 0.90.

Since the precipitation of uric acid as a cuprous salt has been employed as a preliminary step in its determination, it was thought that a similar procedure might be useful for the isolation of the methyluric acids. The urines collected after theophylline administration were pooled and treated as directed in the method of Hunter and Givens (16) for the determination of uric acid. The brown precipitate of cuprous salts was removed by filtration, washed with hot water, and redissolved in weak acid. After the removal of the copper as the sulfide, the filtrate was boiled to remove excess hydrogen sulfide. After concentration to a small volume *in vacuo* and chilling, a yellow orange precipitate was obtained. On further purification approximately 30 mg. of material, light yellow in color, were isolated. Some of this material, as well as uric acid and the three chromogenic methyluric acids, was dissolved in 0.1 per cent lithium carbonate for chromatographic study.

In Solvent A (descending capillarity) the R_F values of uric acid, 1-methyluric acid, 3-methyluric acid, and 1,3-dimethyluric acid, dissolved in lithium

uric acids, had been added. In Solvent B, 1,3-dimethyluric, 1-methyluric,

carbonate, were 0.29, 0.54, 0.67, and 0.91 respectively. In the isolated material, three distinct compounds were present, with R_F values of 0.25, 0.52, and 0.94, corresponding closely to those obtained with pure uric acid, 1-methyluric acid, and 1,3-dimethyluric acid. As a further check, the isolated compound was chromatographed in Solvent B by ascending capillarity. Spots with R_F values of 0.23, 0.36, and 0.63 were obtained. These R_F values again correspond to those for uric acid, 1-methyluric acid, and 1,3-dimethyluric acid, which in this solvent were 0.29, 0.40, and 0.63. From these data it may be concluded that the material isolated from the pooled urine of a rabbit which had received 600 mg. of theophylline in divided doses over a 3 day period contained at least 3 compounds, *i.e.*, uric acid, 1-methyluric acid, and 1,3-dimethyluric acid. After the administration of the first 200 mg. dose, however, only 1,3-dimethyluric acid could be detected by chromatographic analysis of the urine. The ultraviolet absorption spectrum of the isolated material in 0.05 N NaOH was almost identical with that of 1-methyluric acid and 1,3-dimethyluric acid, with a maximum absorption at 295 mμ and a minimum absorption at 260 mμ. If methylxanthines were present in the precipitate, the concentration was too low to be detectable by spectrometric analysis.

After the collection of a 24 hour control urine, a rabbit was given 200 mg. of caffeine by stomach tube. Two 24 hour postfeeding samples of urine were collected. The creatinine excretions of the 3 consecutive days were 130, 133, and 139 mg. respectively. The total chromogens excreted during the same 24 hour periods were 14.2, 20.8, and 26.8 mg. respectively.

A chromatographic study of the three urines, made with Solvents A and B, indicated that all of the urines contained a slow moving chromogen, presumably uric acid, which had an R_F value in both solvents between 0.22 and 0.32. 1-Methyluric acid and 1,3-dimethyluric acid, added to the control rabbit urine, gave average R_F values in Solvent A of 0.69 and 0.89 respectively. The chromatogram in Solvent A of the first 24 hour sample of urine collected after the feeding of caffeine indicated the presence of a chromogen with an R_F value of 0.95 in addition to the slow moving chromogen with an R_F of 0.30. In the urine, collected during the second 24 hours after caffeine feeding, two chromogens with R_F values of 0.89 and 0.68 in addition to the slow moving chromogen were found.

Chromatographic analyses of these urines with Solvent B were also made. In the first 24 hour postfeeding sample of urine a chromogen was found with an average R_F of 0.64. In the second 24 hour postfeeding sample one chromogen with an R_F of 0.68 and a second with an R_F of 0.55 were present. The R_F values of 1,3-dimethyluric acid and 1-methyluric acid added to the control rabbit urine were 0.68 and 0.57, respectively, when Solvent B was used.

From this evidence one may conclude that during the first 24 hour period following the feeding of caffeine to a rabbit the chief metabolic product of this compound, detectable by our method, was 1,3-dimethyluric acid. During the second 24 hour period after caffeine feeding, 1,3-dimethyluric acid, as well as a second chromogen which migrated at approximately the same rate as 1-methyluric acid, was found in the urine. Unfortunately 3-methyluric acid was not added to the control urine of this experiment and chromatographed simultaneously with 1-methyluric acid and 1,3-dimethyluric acid. However, 3-methyluric acid and 1,3-dimethyluric acid added to another 24 hour sample of control rabbit urine gave R_F values of 0.78 and 0.91 when chromatographed in Solvent A. Since this latter R_F value (0.91) is essentially the same as that obtained previously (0.89) for 1,3-dimethyluric acid added to the control urine of this experiment, an R_F of 0.78 for 3-methyluric acid added to the control urine would seem to be a reasonable value. It should also be pointed out that, when the methyluric acids were dissolved in lithium carbonate, the R_F value of the 3-methyluric acid (0.67) was intermediate between that of 1-methyluric acid (0.54) and 1,3-dimethyluric acid (0.91) when chromatographed in Solvent A. The evidence at hand strongly indicates that the chromogen in addition to 1,3-dimethyluric acid is 1-methyluric acid and not 3-methyluric acid.

DISCUSSION

After the ingestion of caffeine, the human subject used in these experiments excreted non-uric acid chromogens, 63 per cent of which were precipitated by ammoniacal silver reagent. In earlier experiments (5) made on three subjects in this laboratory, 80 to 100 per cent of the non-uric acid chromogens, which were excreted after oral administration of caffeine, were precipitated by this silver reagent. 1-Methyluric acid added to normal urine at a level of 110 mg. per liter was recovered to the extent of 87 per cent by the silver precipitation procedure. In contrast, 3-methyluric acid and 1,3-dimethyluric acid, added to normal urine to give a final concentration of 200 mg. per liter, were not precipitated by the ammoniacal silver reagent. These results indicate that the principal chromogenic compound excreted by man after the ingestion of caffeine is 1-methyluric acid. The evidence, obtained by paper chromatography, presented in Table I, supports this view. The data presented in Table I also indicate that the urine collected after caffeine administration may contain some 1,3-dimethyluric acid. If 3-methyluric acid is present, the concentration is too low to be detected by the chromatographic procedure employed by us. Johnson (17) concluded that 1-methyluric acid was present in the urine after caffeine ingestion, but he could not detect 3-methyluric acid. His analytical pro-

cedure, however, was applied to a precipitate obtained by the addition of an ammoniacal silver solution to urine. If, as our work indicated, 3-methyluric acid is not precipitated as the silver salt, it would not be detected, if present, by the procedure of Johnson.

After the ingestion of theophylline by the subject of these experiments, chromatographic studies, with 3 solvents, demonstrated that part of the increase in chromogens was due to 1,3-dimethyluric acid and 1-methyluric acid. No evidence for the presence of 3-methyluric acid was obtained. These results are, in part, in agreement with those of Brodie *et al.* (10) who were able to isolate 1,3-dimethyluric acid from the urine of human subjects collected after the ingestion of theophylline, but who were unable to detect 3-methyluric acid in these urines. These workers stated that approximately one-half of the theophylline was excreted as 1,3-dimethyluric acid, but little, if any, as 1-methyluric acid. In previous work in this laboratory (5) approximately one-half of the increased non-uric acid chromogens excreted by two subjects after the ingestion of theophylline was precipitated by the ammoniacal silver reagent. This fraction, as pointed out previously, is believed to be 1-methyluric acid. The chromatographic results presented here and the report of Johnson (17) give strong support to the view that 1-methyluric acid, in addition to 1,3-dimethyluric acid, is an important metabolic product of theophylline metabolism in man.

The oral administration of theophylline to rats was followed by the urinary excretion of 1,3-dimethyluric acid and 1-methyluric acid. No evidence for the presence of 3-methyluric acid in the urine could be obtained by the method of chromatography. The absence of 3-methyluric acid was to be expected on the basis of the results of Myers and Hanzal (6) who failed to detect an elevation in urinary total chromogens when 3-methyluric acid was administered intraperitoneally to rats. These workers found that 1-methyluric acid administered similarly to rats produced an elevation in urinary total chromogens equivalent to the chromogenic value of the injected material, which suggested that 1-methyluric acid is excreted unchanged. 1,3-Dimethyluric acid augmented the chromogenic value of the urine to an extent which suggested that not only was the compound excreted but some demethylation to 1-methyluric acid had taken place.

The material isolated from the urine of a rabbit which received 600 mg. of theophylline over a period of 3 days contained uric acid, 1-methyluric acid, and 1,3-dimethyluric acid. Uric acid was also present in the control urine collected before the administration of the theophylline. A direct chromatographic study of the 24 hour sample of urine collected after the administration of the first 200 mg. dose of theophylline revealed the presence of only one additional chromogen, which migrated at a rate similar to that of 1,3-dimethyluric acid when it was added to the control urine.

Since 1-methyluric acid, as well as 1,3-dimethyluric acid, was found in the material isolated from the pooled urine collected during the entire period of theophylline administration, this may indicate that the dimethyluric acid is the primary product, which is slowly demethylated at the 3 position. A similar result was obtained when the rabbit was fed caffeine. During the first 24 hour period after receiving caffeine, the rabbit excreted 1,3-dimethyluric acid, but during the second 24 hour period both 1,3-dimethyluric acid and a chromogen which behaves chromatographically like 1-methyluric acid.

Since, per mg., the methyluric acids substituted in the 7 position have a low chromogenic value, the presence of these compounds would not be readily detected by the present methods. It is possible that, after the feeding of caffeine, considerable amounts of the 7-methyl-substituted uric acids are excreted. Johnson (17) has stated that 7-methyluric acid can be detected in the urine of a human subject after ingestion of caffeine. Methods for the quantitative measurement of the methyluric acids substituted in the 7 position are being investigated in our laboratory.

It may be noted that Brodie *et al.* (10) found that when theophylline was given to man only a small part was excreted in unchanged form. Although no data are submitted in the present paper, it is of interest that, in a series of experiments in which rabbits received 190 mg. of caffeine intravenously over a period of 3 days, the maximum excretion of caffeine was 13 per cent of the amount injected.

SUMMARY

After the ingestion of caffeine by a human subject, definite evidence has been obtained that 1-methyluric acid is excreted in the urine. A small amount of 1,3-dimethyluric acid may have been excreted. After caffeine was fed to a rabbit, 1,3-dimethyluric acid and probably 1-methyluric acid were excreted. After theophylline ingestion by man, the rat, and the rabbit, both 1-methyl- and 1,3-dimethyluric acids were excreted.

BIBLIOGRAPHY

1. Benedict, S. R., *J. Lab. and Clin. Med.*, **2**, 1 (1916).
2. Mendel, L. B., and Wardell, E. L., *J. Am. Med. Assn.*, **68**, 1805 (1917).
3. Clark, G. W., and de Lorimier, A. A., *Am. J. Physiol.*, **77**, 491 (1926).
4. Myers, V. C., and Wardell, E. L., *J. Biol. Chem.*, **77**, 697 (1928).
5. Buchanan, O. H., Christman, A. A., and Block, W. D., *J. Biol. Chem.*, **157**, 1[illegible] (1945).
6. Myers, V. C., and Hanzal, R. F., *J. Biol. Chem.*, **162**, 309 (1946).
7. Martin, G. J., *Exp. Med. and Surg.*, **6**, 24 (1948).
8. Buchanan, O. H., Block, W. D., and Christman, A. A., *J. Biol. Chem.*, **157**, 1[illegible] (1945).

9. Benedict, S. R., and Hitchcock, E. H., *J. Biol. Chem.*, **20**, 619 (1915).
10. Brodie, B. B., Axelrod, J., and Reichenthal, J., *J. Biol. Chem.*, **194**, 215 (1952).
11. Weinfeld, H., *Federation Proc.*, **10**, 267 (1951).
12. Consden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.*, **38**, 224 (1944).
13. Williams, R. J., and Kirby, H., *Science*, **107**, 481 (1948).
14. Christman, A. A., and Ravwitch, S., *J. Biol. Chem.*, **95**, 115 (1932).
15. Benedict, S. R., *J. Biol. Chem.*, **51**, 187 (1922).
16. Hunter, A., and Givens, M. H., *J. Biol. Chem.*, **17**, 37 (1914).
17. Johnson, E. A., *Biochem. J.*, **51**, 133 (1952).

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ENHANCEMENT OF HUMAN PERFORMANCE BY CAFFEINE AND THE AMPHETAMINES[1]

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TABLE OF CONTENTS

I. Introduction 1
II. Physical endurance and capacity 2
A. Laboratory studies 2
B. Military field studies 3
C. Athletic performance 5
III. Motor coordination and control 6
A. Reaction time 7
B. Steadiness 9
C. Coordination 10
IV. Monitoring 12
V. Learning 17
VI. Simple and complex verbal and arithmetic tasks 18
VII. Judgment and mood 21
A. Effects on judgment 21
B. Effects on mood 28
VIII. Concluding remarks 30

I. INTRODUCTION

Although many drugs have been used in attempts to enhance performance, only caffeine and the amphetamines[2] have been studied extensively enough to permit a fairly thorough evaluation of their effects. Caffeine is more widely used, being found in coffee, tea, and cola drinks. Amphetamines, at least in this country, are legitimately available only on prescription but enjoy a wide illicit sale.

The main concern of this review is with the effects of these drugs on human performance. We shall also be concerned, however, with the question of whether, in the process of enhancing performance, effects are introduced that are considerably more dangerous and objectionable than low or impaired efficiency.

[1] The present paper is based, in part, on a review written by the authors for Stanford Research Institute Project No. SU-3024, Life Sciences Division, entitled "Drug Enhancement of Performance," and prepared under contract Nonr-2993 (00) for the Physiological Psychology Branch, Psychological Sciences Division, Office of Naval Research. The preparation of the present version was supported by grants MY-3229 and B-865 from the National Institutes of Health, U. S. Public Health Service.

[2] We shall discuss the following compounds under this rubric: Amphetamine Sulfate, U.S.P. [Benzedrine, *dl*-1-phenyl-2-amino-propane sulfate]; Dextroamphetamine Sulfate, U.S.P. [Dexedrine, *d*-1-phenyl-2-amino-propane sulfate]; Methamphetamine Hydrochloride, U.S.P. [Desoxyn, Pervitin, Syndrox, *d*-1-phenyl-2-methyl-amino-propane hydrochloride]. Unless otherwise noted, drugs were administered orally.

Many people believe that the ultimate cost of using drugs in this context far outweighs a temporary gain. This is an important problem which is indissolubly linked to enhancement of performance.

This review will consider the following types of performance: physical endurance and capacity, motor coordination and control, monitoring, learning, performance on verbal and arithmetic tasks, and subjective effects and judgment. Unless it seemed instructive to include them, inadequately controlled studies have been ignored. Discussions of other aspects of the actions of amphetamines as well as effects on performance may be found in reviews by Ivy and Krasno (58), Ivy and Goetzl (57), and Leake (70). A review by Landis (66) contains other information on coffee.

In our concluding remarks we shall discuss two main issues: 1) can caffeine and the amphetamines actually produce superior performance or do they merely restore to a normal level performance degraded by fatigue, boredom, or other influences?, and 2) are the performance-enhancing effects of these drugs counterbalanced by untoward effects to such an extent that their practical use is not feasible or desirable?

II. PHYSICAL ENDURANCE AND CAPACITY[3]

One reason for trying to find agents which enhance performance is that there are physical limitations on how long or how efficiently an individual can continue to perform a task requiring a high rate of energy output. The decline in the capacity to continue such a task or to perform other tasks is what most often is meant by fatigue.

A. Laboratory studies

Although many studies of caffeine were conducted in German laboratories toward the end of the nineteenth century, Rivers and Webber (93) are acknowledged to be the first investigators interested in enhancement of performance to have recognized the necessity for suitable controls, particularly the inclusion of placebo sessions. These authors studied their own performance on a Mosso ergograph (pulling a weight with a finger), under the influence of either caffeine citrate or the inactive agent. The subject was not told which of the two he had been given. A dose of 500 mg of caffeine citrate produced consistent increases in work output.

Foltz *et al.* (30) first worked their four subjects to exhaustion on a bicycle ergometer. Then, after a 10-minute rest, the subjects worked to exhaustion again. Caffeine sodium benzoate (500 mg) given i.v. during the rest period significantly increased work output during the second work period when compared to control injections. In a similar study, Foltz *et al.* (31) compared amphetamine (10 to 15 mg), methamphetamine (5 mg), caffeine sodium benzoate (500 mg), and placebo. The drugs were administered i.v. between 30 seconds and 30 minutes before the first work period. Amphetamine had no detectable

[3] The effects on physical fitness and athletic performance of various pharmacological and nutritive agents have been subjects of three other reviews (12, 49, 75).

effect on work output, methamphetamine produced a substantial effect, and caffeine fell somewhere in between. The large effect of methamphetamine and the small effect of amphetamine, in comparable dosages, is a curious one, for on other tasks, the effects of these two agents are quite similar. However, only two subjects were given amphetamine compared to four with the other drugs, so that the apparent difference could be the fault of sampling error.

These investigators also examined the effects of these agents on the performance of 23 untrained subjects (32). For this experiment, the subject, loaded with a knapsack equal to one-third his body weight, stepped up and down a 16-inch step every 3 seconds. He continued until he could no longer maintain this rate. After a rest, he again worked to exhaustion. On each experimental day a subject worked 3 periods. One hour before the first work period, the subjects took oral doses of amphetamine (10 mg), caffeine sodium benzoate (500 mg), or placebo. No significant differences among treatments emerged. However, the subjects improved so steeply with practice that a true drug effect may have been difficult to discern.

Alles and Feigen found that an oral dose of 10 mg of amphetamine kept performance on a Mosso ergograph from declining (2). Only a higher dose (20 mg) seemed to produce any increase in work capacity above the initial level. Caffeine, in doses up to 400 mg, produced a much less marked effect. Doses of 10 to 15 mg of amphetamine and 10 mg of methamphetamine produced increases in performance on both bicycle and hand ergometers in a well-controlled study by Cuthbertson and Knox (21). Knoefel (63) carried out a study with a bicycle ergometer in which he compared amphetamine, methamphetamine, and the dextro-isomers of both. He reported an increase in work output with 10 mg of methamphetamine and more activity from the dextro-isomer than from the racemic form. Lehmann *et al.* (71) made repeated studies on three subjects who rode a bicycle ergometer to exhaustion. They found the time to exhaustion was prolonged by 5, 10, or 15 mg of methamphetamine compared to placebo trials. Eighteen subjects in a study by Bujas and Petz (13) tried to maintain an 8.5-kg load as a test of static work. A dose of 15 mg of amphetamine 90 minutes before testing produced a statistically significant increase in static endurance compared to the placebo (206 *versus* 186 seconds).

These studies suggest that both caffeine and the amphetamines prolong the amount of time during which an individual can perform physically exhausting work. They also contain occasional hints that these drugs may raise the level of performance above the base line level. Such a small total number of subjects was involved, however, that these results cannot be classed as definitive.

B. Military field studies

Reports during World War II that the Germans were using methamphetamine to prolong endurance in their soldiers prompted the Allied Nations to study the effectiveness of this type of drug in the field. The most extensive series of experiments was conducted under the supervision of Seashore and Ivy (97). In all but two of the studies the subjects were soldiers, and attempts were made to mimic

actual military conditions as closely as possible. Four drug treatments were compared: caffeine sodium benzoate (450 mg); amphetamine sulfate (10 mg); methamphetamine hydrochloride (5 mg); and lactose placebo. The drugs were administered orally in identical capsules.

In the first study, the subjects participated in an all-day, 18- to 20-mile hike followed by guard duty from 6:00 P.M. to 3:00 or 4:30 A.M. Then testing started and lasted 1.5 hours. A first capsule of the drug was given at 6:00 P.M. and a second capsule of the same drug at midnight. The other studies involved training marches with full pack, driving a truck 18 to 20 hours per day, operating a tank for 5 hours per day, performing in hot-moist and hot-dry environments, and marching or operating a tank in the desert. The drugs generally were superior to the placebo in their effects on both subjective symptoms (*e.g.*, sleepiness) and motor tests (*e.g.*, motor coordination), but the effects on the former were more pronounced.

Somerville (103) also fatigued his subjects before testing. In one of his studies, two groups of 50 subjects each marched for 17 hours. One hour before the end of the march, one group received tablets containing 15 mg of amphetamine. The other group received an inert substance. At the end of the march, the subjects were evaluated on an obstacle course and in rifle marksmanship. No differences in the performance of these tasks appeared. In a second study, the subjects underwent a military exercise lasting 56 hours that included both day and night marches. During the last 22 hours, one group took a total of 30 mg of amphetamine, one a total of 35 mg of amphetamine, and the third received only lactose. While differences in the accuracy of rifle firing and times for covering the obstacle course were small, the latter were significantly shorter under the influence of the drug. A third experiment used officers taking part in a War Staff course. These were divided into three groups. One group received 20 mg of amphetamine on two occasions during the experiment; one received lactose; and one received nothing. The subjects had to complete, over a period of 72 hours, a program consisting of 9 exercises in staff duties. During the first 42 hours they did not sleep. The drug did not prevent sleep or improve performance.

Cuthbertson and Knox (21) performed two studies in the field. One involved the effects of 15 mg of methamphetamine on the performance of an 18-mile route march after 24 hours without sleep. No difference was found between the drug-treated and control groups on total time to cover the course. However, a few more of the men given control medication fell out of the march than the men given methamphetamine (7/28 *versus* 3/27). Two companies of infantry served in the second experiment, which lasted 2 days. On the first day, they marched 23 miles, then received 10 or 15 mg of amphetamine or a control capsule before going to sleep. On the second day, they marched 20 miles and "skirmished" with the "enemy" after which the drugs were given. They then "attacked." The amphetamine group slept less well, but was less fatigued in the morning. Seventy percent of the men given drug and 23% of the men given placebo thought the pills helpful. No "hangover" difference was apparent. A mimeographed report by Winfield (111) discusses the administration of amphetamine to RAF bomber

crews on prolonged missions. Winfield's survey demonstrated fewer complaints of fatigue after amphetamine than after placebos.

C. Athletic performance

The most thorough study of the effects of drugs on athletic performance was performed by Smith and Beecher (100).[4] Although 6 experiments were performed, the one in which they were able to impose the most rigid controls and experimental design was the first. The subjects were 15 college swimmers. Each swam his preferred event twice on each of 12 consecutive experimental days. The second swim began 15 minutes after the end of the first. On 4 of the 12 days, each subject received a dose of amphetamine sulfate equal to 14 mg/70 kg body weight; on 4 other days he received a control medication; and on the other 4 days he received a dose of secobarbital equal to 100 mg/70 kg. The interval between the medication and the swim was 2 or 3 hours for amphetamine and 55 minutes for secobarbital. These intervals apparently were based on preliminary studies. The same dose levels were used in the other experiments. The 12 experimental days were grouped into 6 competitive days (3 men per group) and 6 individual days. The events included the 100-yard butterfly, the 100-yard freestyle, the 200-yard freestyle, the 200-yard breaststroke, and the 200-yard backstroke. On the first swim, 14 out of the 15 subjects swam faster with amphetamine than with the placebo. The difference was statistically significant for both the absolute magnitude of the difference and the percentage improvement, which came to a mean of 1.16%. While such a change is small, one must remember, as the authors pointed out, that athletes may practice and train for months to produce such an improvement. (One percent of a 4-minute mile is 2.4 seconds—the difference between fame and oblivion.) On the second swim, only the 100-yard events were improved by amphetamine. When the subjects swam in competition, the drug tended to produce a smaller effect. Secobarbital impaired performance.

Nine track men served as subjects in experiment 2. Three ran 600 yards; 3 ran 1,000 yards, and 3 ran 1 mile. They ran in competitive groups of 3. Eight of the 9 subjects ran faster with amphetamine than with the placebo. The results of experiments 3 and 4, with track events and a marathon run, respectively, were in the same direction. Combining these three studies gave a statistically significant difference in favor of the drug.

Thirteen collegiate weight-throwers and shot-putters took part in experiment 5. Nine threw the 35-lb. weight; four put the 16-lb. shot. Both maximum and mean distances thrown were increased with amphetamine. Maximum distance scores improved 4.36%, while mean distance scores improved 4.39%.

In experiment 6, 16 swimmers each swam 6 times, 3 with amphetamine, 3 with a placebo. This time, the subjects were promised a steak dinner if they equalled or excelled their median competition time for the last 3 meets of the season. Despite the increase in speed evoked by this device, 11 of the 16 subjects

[4] A critique of these studies by Pierson (90a) has been adequately rebutted by Cochran, Smith, and Beecher (19a).

swam faster with amphetamine than with placebo, and the difference was statistically significant when the times for the 100-yard and the 200-yard events were combined.

Karpovich (60) studied running on a treadmill, swimming, and various track events. Neither 10 mg of amphetamine given 1 hour before testing nor 20 mg given 30 minutes before testing led to any improvement in performance. However, Karpovich might have detected improvement if, like Smith and Beecher, he had allowed at least 2 hours to elapse before testing in order to measure the peak effects of the drug [*cf.* Eysenck *et al.* (27) and Franks and Trouton (33)]. Haldi and Wynn (39) also failed to detect any effects on athletic performance. They studied 12 subjects, none of whom was an expert swimmer. Ninety minutes before a swim of 100 yards, the subjects ingested either placebo, 100 mg of metrazol, 5 mg of amphetamine, or 250 mg of caffeine. No drug effects could be found. However, not only was the dose rather small compared to that used by Smith and Beecher, but the use of subjects who were not trained swimmers probably introduced considerably more variability.

There is little doubt, then, that amphetamine can produce a significant enhancement of athletic performance, even in events in which, like putting the shot, one cannot see where endurance or fatigue would play a major role. These conclusions are not negated either by the Karpovich experiment or by the Haldi and Wynn experiment because of the difference in time since administration in the first case and the use of a lower dose and untrained swimmers in the second.

III. MOTOR COORDINATION AND CONTROL

This section deals with studies encompassing a wide range of tasks. They have been brought together because these tasks call mainly for relatively fine motor adjustments instead of gross muscular effort and endurance. Unfortunately, much of the evidence presented in this section is variable and contradictory, and few consistencies emerge. This situation is partly attributable to the neglect in many experiments of the crucial role played by slight variations in method. Small differences among the methods used to assess what is assumed to be a common underlying process, for example, reaction time, can lead to substantial differences in sensitivity to drug effects. In the reaction time study by Carpenter discussed below (16), for instance, the detection of an effect of caffeine depended on the brightness of the visual stimulus. Moreover, it also depended on dose level. The interaction of performance parameters and dose level is rarely assessed. Most of the investigations reported in this section employed one arbitrarily selected set of performance parameters, one dose level of the drug, and a single time of testing after drug. Without information about the interactions of these variables, the conclusions that we present must be regarded, for the most part, as provisional.

These conclusions may be listed as follows: 1) Caffeine has little or no effect on reaction time, whereas amphetamine seems to lower it, especially in fatigued subjects. 2) Caffeine impairs hand steadiness, whereas amphetamine seems to improve it. 3) Caffeine has equivocal effects on coordination, and amphetamine improves such performance, especially the more complex ones. 4) Both caffeine

and amphetamine can, to some extent, counteract decrement of motor performance produced by alcohol.

The individual studies are discussed below.

A. Reaction time

The effects of caffeine on reaction time are in dispute. Cheney (18, 19) found discriminative reaction time to a series of lights reduced by 4% after coffee (1 cup) and 8% after caffeine (180 mg, 2 hours before the test). The number of incorrect responses also was reduced. Unfortunately, the description of the experiment implies that every subject received the treatments in the same order—a procedure which makes it difficult to interpret these findings. Horst and Jenkins (55) reported a decrease in simple reaction time after the ingestion of 3 or 4 mg of caffeine/kg, as did Gilliland and Nelson (34) after either coffee alone or coffee plus added caffeine. Unfortunately, the use of coffee does not permit a precise statement of the amount of caffeine ingested. Moreover, the data with caffeine are then confounded with a mode of administration that contributes unique effects of its own unless the appropriate controls are included.

Lengthened reaction time after caffeine was reported by Hollingworth (52), Schilling (96), and Hawk (47). The last author had his subjects drink from 2 to 6 cups of coffee per day and found, when coffee drinking was terminated at the end of several weeks, that reaction time rose. This might have been the result of withdrawal, but the report is of only abstract length and not enough details are available to make a more definite statement. Schilling used an auditory reaction time task and administered 300 mg of alkaloidal caffeine 15 minutes before a 35-minute period during which reaction time was sampled every 5 or 10 minutes. While the author claimed an increase in reaction time after caffeine relative to placebo, our statistical analysis of his raw data gives a $t = 1.3$, which is not significant. Also, as shown by Hollingworth (52) and others, 50 minutes is probably too short a latency for peak drug effects to appear when caffeine is taken orally.

The most thorough study on caffeine was done a half century ago by Hollingworth (52). The care with which he devised appropriate controls has rarely been exceeded in contemporary research. Hollingworth found a dose-related effect on reaction time; smaller amounts of alkaloidal caffeine (60 to 240 mg) produced longer reaction times than placebo. Actually, Hollingworth says, the initial effect of the drug is a "briskness" which leads to false reactions, and the retardation in reaction time possibly follows because the subject becomes wary. Higher doses (300 to 360 mg) decreased reaction time within 2 hours. Hollingworth thought that the retardation seen with smaller doses was overcome by the extreme stimulation associated with these higher doses.

No differences between control medications and caffeine appeared in the reaction time studies of Thornton *et al.* (106), Adler *et al.* (1), and Seashore and Ivy (97). Lehmann and Csank (72) could demonstrate no shortening of simple reaction time after 600 to 900 mg of caffeine citrate. Thus, one is led to the

conclusion that caffeine, in ordinary doses, has little effect (or at least an inconsistent effect) on either simple or complex reaction time.

There also appears to be little effect of caffeine on the increase in reaction time produced by certain doses of alcohol. In a well-designed study, Carpenter (16) administered alcohol in doses of 0, 0.4, and 0.8 ml/kg to 9 subjects. Fifteen minutes were allowed for drinking. Capsules containing placebo, 1.47 mg caffeine/kg, or 2.94 mg caffeine/kg were ingested at the beginning of the drinking period. The first reaction time test (11 trials) began 30 minutes after ingestion of capsules. Subsequent tests followed every 10 minutes until 80 minutes post-ingestion. The reaction time apparatus presented a visual stimulus of either low or high intensity, and the subject responded by pressing a button. Alcohol increased reaction time in proportion to dose level. Caffeine had no over-all effect on reaction time. It did, however, increase the difference produced by variation in stimulus intensity. The data also suggest that caffeine lowered reaction time at the higher doses of alcohol when the high-intensity stimulus was used. The interactions shown in these results emphasize how much information is lost when experimenters arbitrarily select single values of performance parameters and drug dosages.

The effects of amphetamine are somewhat more consistent. Adler *et al.* (1) found a decrease in discriminative reaction time after 10 mg of *d*-amphetamine, even at a simulated altitude of 18,000 feet. Thornton *et al.* (106) found a small, not significant decrease in reaction time after amphetamine, but their experiment involved only 3 subjects. Lehmann and Csank (72) produced a marked shortening of simple reaction time after rather high doses (12.5 to 15 mg) of *d*-amphetamine. Goldstein *et al.* (35) observed no effect. They administered 10 mg of *d*-amphetamine 60 minutes before tests of both simple and discriminative reaction time. However, their results are confounded with a large practice-effect. The negative results of Rasch *et al.* (91) after 20 mg of amphetamine could also be due to confounding with a practice-effect, but their description of the experiment is too unclear for one to be sure. The above studies used non-fatigued subjects. From them we would conclude that amphetamine tends to reduce reaction time. This effect was also seen in fatigued subjects. Kornetsky *et al.* (65) found that 15 mg of *d*-amphetamine administered 90 minutes before testing eliminated the increase in discriminative reaction time engendered by 68 hours without sleep. Seashore and Ivy (97), also using fatigued subjects, found a significant decrease in discriminative reaction time following the administration of 10 mg of amphetamine or 5 mg of methamphetamine. The subjects had been awake for 24 hours at the time of testing. Tyler (109) reported that the deterioration in reaction time which occurred after 60 hours of sleep-loss was counteracted by amphetamine. The only study that showed no effect on the reaction time of fatigued subjects was the one by Cuthbertson and Knox (21). Thus, most of these studies indicate that reaction time lengthened by fatigue can be restored with amphetamine. Again, it must be underscored that differences in method probably contribute a great deal to the inconsistencies in the data.

B. Steadiness

Caffeine seems to impair the ability to maintain the arm in one position without tremor, while amphetamines seem to improve such ability slightly. Hollingworth (52) required the subjects to hold a metal stylus in a hole without touching the sides. He observed a pronounced unsteadiness that reached its peak about 3 to 4 hours after the administration of 360 mg of caffeine. Other investigators have confirmed these results. Thus, Hull (56) found increased tremor after 300 mg of caffeine citrate. Steadiness was also impaired after 300 mg of caffeine sodium benzoate (106), or after a cup of coffee (34). Adler *et al.* (1) found increased hand tremor after 420 mg of caffeine sodium benzoate. Switzer (104, 105) reported a 41 % increase in hand tremor 4 hours after 300 mg of caffeine citrate, and Lehmann and Csank (72) noted a significant decrease in steadiness after 600 to 900 mg of caffeine citrate. Only Seashore and Ivy (97) reported no effect on steadiness, but their subjects were fatigued, and this may account for the difference.

Although they found no effect in fatigued subjects on the hole-and-stylus steadiness test with 10 mg of amphetamine or 5 mg of methamphetamine, Seashore and Ivy (97) detected a significant improvement from these agents on arm-hand tremor and sway. They measured tremor and sway by having the subject hold a small ball attached to horizontal and vertical threads which operated ratchet mechanisms to give counts of movements in 4 directions. Postural sway, with the subject standing erect, also decreased after amphetamine and methamphetamine. Another study on fatigued subjects reported an improvement in steadiness and body sway after amphetamine (109). In non-fatigued subjects, *d*-amphetamine had no effect on body sway (28). A dose of 10 mg was followed 4.5 hours later by a dose of 5 mg. Thirty minutes later body sway was measured.

Both Thornton *et al.* (106) and Adler *et al.* (1) found with the stylus-hole method that amphetamine improved steadiness. Thornton *et al.* gave 20 mg of amphetamine, while Adler *et al.* obtained their results with 10 mg of amphetamine or 10 mg of *d*-amphetamine, both at ground level and at a simulated altitude of 18,000 feet. Methamphetamine (5 mg) had little effect under the latter conditions. Balloch *et al.* (5) found no effect from either 10 or 20 mg of *d*-amphetamine, nor did Goldstein *et al.* (35) after 10 mg of *d*-amphetamine.

Hauty (40) attempted to separate two components of hand steadiness in order to see whether they were differentially susceptible to drugs; these were high frequency, low amplitude tremor, and low frequency, high amplitude compensatory movements—what could be called "drift." The apparatus consisted of a stylus and a series of holes varying in size. The drug treatments consisted of 5 mg of *d*-amphetamine, 10 mg of *d*-amphetamine, 100 mg of secobarbital, 200 mg of secobarbital, placebo, and a no-drug control group. The results showed a significant difference between drugs when the smaller apertures were used; there was essentially no difference among drugs with the larger apertures. At the small ones, both doses of secobarbital tended to reduce tremor while both doses of

d-amphetamine tended to increase it. Only the increase relative to the no-drug group was statistically significant, however; the placebo group, interestingly enough, also demonstrated increased tremor. This is a useful and informative study because it explored both performance and pharmacological parameters to give us a notion of how they interact. It suggests, too, that the discrepancies among the various studies dealing with amphetamine may be due in part to the apparatus used to measure steadiness.

Newman and Newman (82) studied the effect of *d*-amphetamine and caffeine on disturbances of equilibrium and steadiness induced by alcohol. Sixty-six subjects ingested doses of ethyl alcohol equivalent to approximately 0.18 g/kg body weight in a concentration of 20%. This dose was repeated at intervals of 20 minutes through the experiment. When drugs were given, they were taken by mouth 45 minutes before the experiment. The doses were 15 mg of *d*-amphetamine and 300 mg of caffeine. The criteria used were balancing on one foot with eyes closed; hand steadiness; electroencephalogram (EEG); critical flicker frequency. Results were given in terms of the blood alcohol concentration at which "failure" occurred. The drugs had no effect on this concentration. Unfortunately, the authors seem to have given each subject the same order of treatments: alcohol first, alcohol plus *d*-amphetamine second, and alcohol plus caffeine third. This makes it difficult to interpret the data.

C. Coordination

The study of the complex muscular adjustments involved in performing skilled motor tasks is important in assessing the effects of a drug not only for intrinsic empirical value, but because the effects on such tasks may tell a great deal about how a drug acts. In this section, we shall discuss coordination tasks that range in complexity all the way from tapping a finger or a stylus to tracking a moving target.

Hollingworth (52), Horst *et al.* (53, 54), Thornton *et al.* (106), and Lehmann and Csank (72) observed an increased rate of tapping after caffeine. Neither Adler *et al.* (1) nor Flory and Gilbert (29) could find any effect of caffeine on tapping rate, while Gilliland and Nelson (34) claimed a slight increase. The effect of caffeine on this particular response is, therefore, equivocal, probably because the effect depends upon the specific technique employed to measure tapping rate, *e.g.*, whether the subject uses a stylus or his finger, whether he strikes alternate targets or a single one, *etc.* The same may be said about the effects of amphetamine. Thus, Adler *et al.* (1) produced no significant change in tapping rate with amphetamine, *d*-amphetamine, or methamphetamine nor did Balloch *et al.* (5) with *d*-amphetamine. No rise in the rate of working a thumb-operated manual counter followed a dose of 10 mg *d*-amphetamine in a study by Goldstein *et al.* (35). On the other hand, Simonson and Enzer (99) reported a rise with amphetamine but did not indicate the dose used. Lehmann and Csank (72) also noted an increased rate with *d*-amphetamine.

Hollingworth (52) also used a more complex test of coordination in which the subject had to insert a stylus successively into each of three holes. Here 60 or

120 mg of caffeine produced a slight decrease in the time required to complete the task; 180 or 240 mg seemed to produce an increase, and 360 mg seemed to produce a slight initial increase followed by a decrease. A similar task was employed in the study by Goldstein *et al.* (35). A dose of 10 mg of *d*-amphetamine did not change the rate at which subjects simultaneously touched, with a pair of rods, two pairs of disks. An interesting attempt to observe natural repetitive, coordinated movement was made by Golla *et al.* (37). They reported that 20 mg of amphetamine increased the speed of walking across a room and writing one's signature. This experiment, unfortunately, did not incorporate the controls necessary for a clear interpretation of the data. For example, the observer was aware of the drug that had been given.

One type of coordination task requires subjects to follow a moving target or to compensate for movement of a target; this is the skill called tracking. Horst *et al.* (53, 54) required their subjects to strike a pendulum at such a time and with such a force that it fell within a designated area. Caffeine impaired this performance by a considerable amount in doses of 3 to 4 mg/kg when the drug was given every day. Performance remained impaired for a few days after the caffeine regimen had ceased. A decrease in pursuit rotor performance (following a moving target on a turntable with a stylus) that occurred at simulated altitudes of 18,000 feet could be counteracted by amphetamines (1). *d*-Amphetamine, in fact, even with low oxygen pressure, improved performance beyond ground level control scores. Eysenck *et al.* (27) also employed the pursuit rotor. A dose of 10 mg of *d*-amphetamine administered 250 minutes beforehand produced a considerable increase in time-on-target score relative to placebo. An equivalent dose given 75 minutes beforehand produced a significantly smaller enhancement. This latency difference confirms Smith and Beecher's data (100).

One of the tests given fatigued subjects in the Seashore and Ivy (97) experiment required them to keep in level flight a model airplane that continually drifted off-course. All drugs used (caffeine sodium benzoate, 450 mg; amphetamine sulfate, 10 mg; methamphetamine, 5 mg) improved performance relative to the control agent, with the amphetamines proving somewhat more effective than caffeine. Newman (81) used the same task in two experiments. In the first, he compared a placebo and 10 mg of amphetamine given 1 hour before the start of a 1-hour run. No difference due to drug was found. Unfortunately, every subject underwent the same order of treatment: first placebo, then amphetamine. In the second study, the subjects were first deprived of sleep for 36 hours. After a 1-hour control run, they were all given 10 mg of amphetamine and, after a 1-hour test, worked again for 1 hour. Performance after drug was considerably better than pre-drug performance. But, in the absence of suitable controls—the author's failure to consider the effects of order of events—his conclusion that amphetamine produces effects on this task only in fatigued subjects must be regarded as questionable.

Using a simulated automobile driving device, Graf (38) gave his subjects a series of tests every 2 hours, starting at 6:00 P.M. and continuing until noon of the following day. Within each series, several road speeds were tested. The drug

preparations (6 mg of methamphetamine or 200 mg of caffeine) were administered at 1:00 A.M. Data were given for only one subject, and show a maximal effect 3 hours after administration, with methamphetamine producing both a greater and more prolonged action than the caffeine.

A combination of 10 mg of amphetamine and 100 mg of secobarbital sodium has been assessed by Laties (69) in a group performance situation (4 men per group) employing both sleep-deprived and non-sleep-deprived college men. The sleep deprivation period lasted approximately 36 hours. This combination of drugs significantly enhanced performance on both of the tasks employed. These required the subjects to work together in order to succeed. In one, the subjects tried to roll a ball up an inclined spiral plane that was mounted on a movable platform gripped by all four subjects. In the other, the subjects held a frame to which was attached an irregularly bent wire that they attempted to move through a small stationary ring. Performance on an individual task, a one-man version of the group ring-and-wire task, was also improved by the combination of drugs, making it less likely that factors peculiar to the group situation were responsible for its effectiveness.

The data available on coordination allow one to draw the provisional conclusion that the amphetamines can improve this kind of performance, with positive results more likely to appear when the task is complex rather than simple.

There are also reports in the literature of attempts with amphetamine or caffeine to combat the motor deterioration produced by sufficiently high doses of alcohol. Rutenfranz and Jansen (94) studied the effects of ethyl alcohol in doses of 0.5 g/kg and 1.0 g/kg in 2 subjects working on a task which simulated automobile driving. The performance decrement produced by the lower dose of alcohol was only partly counteracted by 200 mg of caffeine, but completely counteracted by 9 mg of methamphetamine. Such a dose of methamphetamine, however, could only partly counteract the decrement produced by the high dose of alcohol, an amount equal to about 6 ounces of 100-proof whiskey.

IV. MONITORING

As more and more human functions are appropriated by mechanical and electronic devices, more and more human activity shifts from detailed physical manipulation of the environment to making merely simple responses to the data conveyed by the device. The class of behavior that subsumes this sort of activity is called monitoring—a term coined by the British.

The number of experiments that have studied drugs in this context is relatively small. But a fairly definitive answer can be given, thanks to a brilliant series of studies carried out at the USAF School of Aviation Medicine at Randolph Field. These studies were aimed at the problems of performance decrement in prolonged monitoring tasks that also included a skill factor. The inclusion of a skill factor makes the task less pure as a monitoring task, but the experimental design and the variables studied make it more reasonable to call these experiments on monitoring rather than experiments on skill. Payne and Hauty (85) and Hauty and Payne (48) already have reviewed some aspects of this work. The task these

investigators chose as their tool was devised by Loucks in World War II for aviation cadet selection (Melton, 76). Called the USAF SAM Multidimensional Pursuit Test, it is based on an airplane-type cockpit in which a subject monitors a display of 4 dials. The dial pointers drift randomly from the null position. The subject's task is to restore the pointers to the null position and maintain them there concurrently by appropriate movements of a joy stick, rudder pedals, and throttle lever. The subjects in these studies were young airmen in basic training. The experimental procedure involved a training period of 50 minutes during which most subjects attained a stable level of proficiency (so that the skill factor did not enter into later performance), a 10-minute rest period for the introduction of drugs and other experimental treatments, and a final work period of several hours. During this final period most subjects showed a progressive decline in the amount of time that the 4 pointers were kept concurrently at the null position. [Sample performance curves are given in Payne and Hauty (85).]

In their first study [Payne and Hauty (84)] the investigators administered 5 treatments before a final work period of 4 hours. These were: no drug, lactose placebo, 5 mg of *d*-amphetamine, 20 mg of a caffeine derivative, and 0.65 mg of scopolamine plus 50 mg of diphenhydramine. *d*-Amphetamine completely arrested the usual decline in performance level with time; in fact, it raised performance above the level reached at the end of the 50-minute practice period. The caffeine compound also reduced performance decrement significantly, but not as strikingly as the *d*-amphetamine. The scopolamine-diphenhydramine combination significantly impaired performance.

A second experiment (41) was designed to investigate the single and joint effects of drugs, cues to pointer position, and statements about the length of the work period. The drug treatments comprised no drug; placebo; 5 mg of *d*-amphetamine; a slow-release dose (spread over 4 hours) of 6.3 mg of *d*-amphetamine sulfate; caffeine derivative (dose not specified); a combination of 5 mg of *d*-amphetamine, 50 mg of diphenhydramine, and 0.65 mg scopolamine; and 50 mg of diphenhydramine plus 0.65 mg of scopolamine. One-third of the subjects were given an auditory signal when all the pointers were at null; one-third were given a visual signal; and one-third had to rely on the usual method—visual scanning of the instrument panel. Half the subjects were told that they would perform for 4 hours, then be given a break. The other half were told that they would perform for 7 hours. All subjects worked for 7 hours, with a 15-minute rest at the end of 4 hours. The shorter goal proximity (4 hours *versus* 7 hours) resulted in better performance, even after the break at the end of 4 hours. An added auditory signal was more effective than an added visual signal, and both were better than the usual procedure.

The results with drugs were independent of the effects of additional cues. *d*-Amphetamine counteracted the usual decline in performance for the full 7 hours. It did not matter whether the drug was given as a single 5-mg capsule or in a sustained-release form. The caffeine derivative was also more effective than the placebo, but failed to match the amphetamine effect. Both of the mixtures containing scopolamine and diphenhydramine led to significantly worse per-

formances than did the placebo, but the mixture that contained the *d*-amphetamine excelled the one lacking it. An interesting difference between the effects of the stimulant drugs and the effects of the added cues appeared in this experiment. While both raised the level of performance, they did so in different ways. The supplementary signals merely raised performance without affecting the rate at which it fell off with time. The effective drugs, however, particularly *d*-amphetamine, exerted their effect mainly by arresting the decline in proficiency as the session progressed.

The same sort of dissociation between the effects of psychological and pharmacological treatments appeared in the third study of the series (86). Here the authors assessed the effects of two psychological variables. One was the amount of information conveyed about the position of the dial pointers—what the authors called "directive feedback." Three levels were imposed: no supplementary cue, a single peripheral visual signal (a light) whenever any one of the instrument settings drifted from null; and, one supplementary signal per instrument (4 lights). The other psychological variable was called motivation. Three levels were specified. In one, the control level, no supplementary cues about the effectiveness of performance were supplied. In the second, the score on the previous block of trials was posted on a pegboard. In the third, the subject could follow his progress through the entire session *via* the pegboard. The following medications were administered: a mixture of 0.65 mg of scopolamine plus 50 mg of diphenhydramine; 5 mg of *d*-amphetamine; placebo. A no-drug group was also included.

The more precise the supplementary cues and the more thorough the information about the subject's performance, the greater was his score. The drug treatments also produced the expected results: *d*-amphetamine arrested the decline in performance with time, while the scopolamine-diphenhydramine mixture accelerated it. As in the previous experiment, the *d*-amphetamine and the psychological variables exerted their benefits in different ways, the latter raising performance level but not changing its rate of decline, the drug counteracting the fall in performance. It is important to note how these results conflict with the hypothesis (see discussion) that amphetamine exerts its benefits simply by making the individual more highly motivated toward the task.

Payne *et al.* (87) then investigated the effect of *d*-amphetamine at various dose levels as a function of the time of administration during the work period. They studied dose levels of 1.25, 2.5, 5.0, 7.5, and 12.5 mg of *d*-amphetamine. These were given at the start, 1 hour after the start, or 2 hours after the start of a 4-hour work period. Two values were computed: the effect on loss of proficiency and the effect on gain of proficiency. Loss was taken to be the difference between the score on the last 30 minutes of the pre-experimental period (the practice period) and the score during the second hour after the drug. A plot of loss *versus* dose gave a quadratic function which showed that the larger the dose, the lower the loss, the asymptote of the function being reached at about 10 mg, and with no loss in performance detectable with doses of 7.5 mg and above. The interaction of dose and delay was not significant. A specified dose had the

same effect no matter when during the session it was given. Gain was taken to be the difference between the score during the last 30 minutes prior to the drug, and the score during the second hour after the drug. Here, too, the function was quadratic. Beyond about 5 mg, no significant additional benefit was realized; there is even a hint in the data of a decrement in gain beyond 10 mg.

The success of *d*-amphetamine in counteracting performance decrement led Payne and Moore (88) to examine other stimulant and depressant drugs with the same technique. We shall discuss only the stimulants, which were: *d*-amphetamine, 5 mg; mephentermine, 25 mg; and pipradrol, 2 mg. The work period lasted 4 hours. *d*-Amphetamine produced the best performance followed closely by mephentermine. Pipradrol at this dose level maintained performance at the pre-drug level throughout the session but did not generate the considerable increase in proficiency attained with *d*-amphetamine and mephentermine.

Up to this point, the work periods had not exceeded 7 hours. What effects would amphetamine produce on performance prolonged considerably beyond this length? Does it engender a "let down" when it wears off? Can it restore performance degraded by extremely prolonged work? An attempt to answer these questions was made by running 24 subjects for 30 continuous hours each (44). The subjects were divided into 3 drug treatment groups, the first medication being given after 1 hour of practice: 1) placebo at 9:00 A.M., day 1, and at 9:00 A.M., day 2; 2) placebo at 9:00 A.M., day 1, and 5 mg of *d*-amphetamine at 9:00 A.M., day 2; 3) 5 mg of *d*-amphetamine at 9:00 A.M., day 1, and 5 mg of *d*-amphetamine at 9:00 A.M., day 2. All three groups performed at a fairly steady level from 9:00 A.M. to midnight on day 1. No decline in proficiency was observed during this time. What was responsible for this lack of decrement is not known. At any rate, the first dose of amphetamine produced no effect on performance. From midnight to 6:00 A.M., performance fell steeply. During this time many subjects had hallucinations. (One saw a little man in a sombrero, holding an umbrella overhead, in the rpm dial. Another believed himself "at the end of a long dark alley walled by tall buildings leaning at crazy angles.") The occurrence of hallucinations was not related to whether amphetamine had been administered the morning before. At 6:00 A.M., performance picked up again, but fluctuated erratically, and, in the group given a placebo at 9:00 A.M. this second morning, never reached the proficiency levels of the day before. In the groups given *d*-amphetamine at 9:00 A.M. of day 2, performance returned to the high initial level of the preceding day after about an hour. No evidence of "let down" in the groups receiving amphetamine was detectable.

Two other experiments by the Randolph Field group (45, 46) were devoted to the study of oxygen deficiency. The first experiment (45) was designed to answer 3 questions. 1) Do normal oxygen levels restore work proficiency lowered by prolonged work and oxygen impoverishment? 2) Are the effects of amphetamine and normal air restoration additive in restoring proficiency? 3) How well will amphetamine sustain performance during interpolated periods of low oxygen? The subjects, after the usual training period, worked for 2 hours on the Multidimensional Pursuit Test while breathing a nitrogen-oxygen mixture containing

12% oxygen. They then worked for 1 hour while breathing 21% (normal) oxygen, and, finally, 1 hour again on 12% oxygen. The drug treatments—lactose placebo or 5 mg of *d*-amphetamine—were given before the start of the 4-hour work period. Amphetamine completely mitigated the marked decrement produced by oxygen impoverishment, except for the first hour after administration when the drug was in the process of being absorbed. Restoration of normal air only slightly arrested the decline in performance of the placebo group and had almost no effect on the amphetamine group. When put back on 12% oxygen, both groups declined at about the same rate.

In the second experiment (46) these investigators studied three different concentrations of oxygen: 60, 21, and 12%. Again, a lactose placebo or 5 mg of *d*-amphetamine was given before the start of a 4-hour work period. *d*-Amphetamine improved performance about equally at all concentrations of oxygen. Performance was better with 60% oxygen than with 21% oxygen, while performance with 12% oxygen was worse than with 21% oxygen. Only the latter difference, however, was statistically significant.

The conclusions that emerge from this series of studies are these: Amphetamine can sustain a high level of proficiency, can restore performance that has deteriorated, can counteract the effects of oxygen deficiency, and contributes something above and beyond what can be achieved with good human engineering design and high motivation. Moreover, it does this without engendering objectionable side-effects.

These conclusions are buttressed by other studies of monitoring. One of the earliest workers to study this type of behavior was Mackworth (73), whose well-known monograph contains an experiment in which his Clock Test was used to assess the action of amphetamine on vigilance. The apparatus consisted of a black pointer which rotated in steps. The pointer moved to a new position once every second, one hundred steps completing one revolution. No scale markings or reference points appeared on the white background. At irregular intervals—12 times in a 30-minute period—the pointer moved two steps instead of one. The subject's task was to press a key whenever a double-step occurred. After the first 30 minutes on this task the incidence of missed signals tended to rise from about 15% to about 25%, where it remained for the rest of the usual 2-hour test period. A dose of 10 mg of amphetamine 1 hour before the session completely counteracted the rise in missed signals. Moreover, the subjects responded more quickly when under drug to the signals they did detect. According to Holland's analysis of the vigilance situation (51), the probable effect of amphetamine in Mackworth's situation was a rise in the frequency of observing responses—looking more frequently at the clock. In tasks such as this, and in those used in the Randolph Field studies, the crucial element in proficiency is steady scanning of the "target." When scanning rate goes down [as it certainly did between midnight and 6:00 A.M. in the 30-hour study of Hauty and Payne (44), since the subjects began to snatch short periods of sleep] then performance falls too.

Two studies on a monitoring type of task by Kornetsky *et al.* (65) and Townsend and Mirsky (107) illustrate how, with some tasks, amphetamine may produce differential effects depending on whether this behavior is normal or impaired.

The task was built around an endless-belt filmstrip that presents letters to the subject at the rate of 1 per second. In one version of the task, the subject is required to press a response key whenever the letter *x* appears. In another version, he presses the key only when the *x* follows an *a*. Townsend and Mirsky (107) could detect no effects of 5 or 15 mg of *d*-amphetamine on either version when the drug was given 135 minutes beforehand. But Kornetsky *et al.* (65), who used subjects deprived of sleep for 68 hours at the time of testing, found significantly fewer missed signals with 15 mg of *d*-amphetamine on both versions. He administered the drug 90 minutes before testing.

V. LEARNING

Drugs can contribute to efficiency not only by maintaining or improving ongoing performance but by speeding the rate at which new behavior is acquired. The question of whether drugs that enhance performance can also hasten learning is an interesting one. It is somewhat surprising to find no more than a handful of studies devoted to it.

Switzer (104) observed the effects of caffeine on "inhibition of delay." The conditioned stimulus (CS) was a 21-second light. The unconditioned stimulus (US) was an electric shock applied for 1.2 seconds at the end of the 16th second of the CS. The galvanic skin response (GSR) served as the measure of conditioning. The subjects in the study received 300 mg of caffeine citrate or a lactose placebo 4 hours before a session. A session was run on each of 5 experimental days. Administration of caffeine resulted in a significantly shorter response latency to the CS than did the placebo (9.84 *versus* 11.72 seconds); that is, the rise in GSR amplitude during the CS occurred earlier after caffeine. The drug also produced a significantly greater amplitude of response.

To investigate extinction, Switzer (105) used a CS (light) that lasted 0.95 second. The US, electric shock, overlapped the CS by 0.15 second and continued for 1.05 seconds following the termination of the CS. Capsules of either caffeine citrate (300 mg) or lactose placebo were ingested 4 hours before a session. On each of the 4 days when an extinction session was run, the subject underwent a series of unreinforced CS's followed by 1 or 2 CS-US pairings. Even during extinction caffeine increased the amplitude of the GSR.

Hull's (56) subjects learned nonsense syllable lists, each of which was 16 syllables long, by the anticipation method (giving the next item in the series). After taking 300 mg of caffeine citrate or a lactose placebo, the subjects relearned two lists from the previous day, then, starting 3.5 hours after ingestion of the drug, learned two new series to a criterion of 2 perfect repetitions. Caffeine significantly increased the number of anticipatory intrusions. These were defined as giving, instead of the correct word, a word that came later in the series. The length of anticipatory displacements (the number of syllables skipped) also increased significantly. Despite this, caffeine affected neither the number of trials needed to attain the criterion, nor retention.

The experiments by Switzer (104, 105) and by Hull (56) suggest that caffeine does not affect either the development or extinction of a response, but simply provokes a tendency to respond early or more strongly. Hollingworth's (52)

observation is in accord with this view. He attributed the lengthened reaction time found after moderate doses of caffeine to the subjects' attempts to inhibit quick, false reactions. The data available do not allow one to make any further statements about caffeine and learning.

The data on amphetamine are also sparse. Franks and Trouton (33) investigated the effects of *d*-amphetamine on eyeblink conditioning. A puff of air served as the US and a tone as the CS. Eyelid movements were recorded photoelectrically. The drug treatments consisted of sodium amobarbital (270 mg), *d*-amphetamine (10 mg), or placebo 45 minutes before the start of the session, or 10 mg of *d*-amphetamine 2 hours before the start. The subjects given *d*-amphetamine 2 hours before the experiment became conditioned more quickly than the placebo group, while the amobarbital group became conditioned less quickly. The group given *d*-amphetamine 45 minutes before conditioning did not differ from the placebo group. [This latency of effect confirms the statement by Smith and Beecher (100) that 2 to 3 hours are required to attain the peak effects of such a dose level.] It would have been useful to have observed whether the groups differed after the effects of the drugs had worn off, because then one could have said whether the effects on conditioning were permanent or whether they were transitory and reflected merely, perhaps, a greater responsiveness to the conditioned or unconditioned stimuli.

d-Amphetamine (5 or 15 mg) given 90 minutes before testing did not affect the ability of rested subjects to learn which of 10 stimulus-lights were connected to which of 10 response-buttons (64). The subject had to press the appropriate button when a light flashed. In subjects deprived of sleep for 68 hours before testing, 10 mg of *d*-amphetamine at 44 hours and 15 mg at 68 hours significantly improved both time and error scores (65). The data reported by Eysenck *et al.* (27) of pursuit rotor performance indicated that the subjects given 10 mg of *d*-amphetamine 250 minutes before the session rose in proficiency at a greater rate than those given a placebo or 10 mg of *d*-amphetamine 75 minutes before the session. If the relevant variable were not a drug, most psychologists would not hesitate to call this an increase in rate of learning. Under the circumstances, however, one is justified in calling for more fundamental evidence before accepting such a conclusion. Simply promoting a greater rate of improvement in proficiency is insufficient proof, in view of the numerous effects of amphetamine on motor performance. A more convincing experiment would be one in which the increased rate of gain in proficiency was carried over to a subsequent occasion.

Amphetamine, then, seems to hasten conditioning, to restore in part the degraded rate at which a new discrimination is learned by sleepy subjects, and to increase the rate at which subjects acquire proficiency in a motor skill. But there are no data to answer the more important question of whether these effects are permanent or transient.

VI. SIMPLE AND COMPLEX VERBAL AND ARITHMETIC TASKS

A variety of tasks explicitly designed to assess verbal and arithmetic behavior has been studied by investigators interested in drug effects. These range from

crossing out numbers or letters to standard intelligence tests. Neither the results of the simple nor the results of the complex tasks offer much hope of an affirmative answer to the question: "Can drugs help to raise the level of 'intellectual' performance in normal subjects?"

Barmack (9) found that 120 mg of caffeine increased the number of addition problems attempted, the same effect he had found previously with 10 mg of amphetamine (6). Gilliland and Nelson (34) also reported that the ingestion of one or two cups of coffee 100 minutes previously increased the rate of adding. The subjects in the experiment by Kleemeier and Kleemeier (62) showed significant increases in rate of addition and multiplication after 10 mg of amphetamine. McNamara and Miller (74) used written multiplication problems to measure the effects of 20 mg of amphetamine given 2 hours previously. Neither the number of problems attempted in a 12-minute period nor the number of errors was altered by the drug, but the presence of a substantial practice effect makes this result ambiguous. A dose of 10 mg of *d*-amphetamine produced no effect on rate of addition in the study by Goldstein *et al.* (35). In the test used by Hollingworth (52), the subjects had to add *17* to a number on a card and give the answer orally. The time required to perform 50 calculations consistently decreased after caffeine over a wide range of dose levels (60 to 360 mg). Graf's (38) subjects worked arithmetic problems for 3 continuous hours. At 60 and 120 minutes after the beginning of the session, solutions of the drug were administered by mouth. On methamphetamine days the dose was 3 mg per administration. On caffeine days the dose was 100 mg per administration. (It is not clear whether a placebo was given on control days.) After a 90-minute pause for lunch, there were three more 30-minute work periods separated by 30-minute rest periods. The rate of addition fell off less on caffeine days than on control days, but methamphetamine led to a substantial rise in rate above the control level. Thus, there is some evidence that caffeine and amphetamine can improve performance on arithmetic tasks, especially if the experimental sessions are long.

Compensation for the effects of alcohol has also been tested with arithmetic problems. Graf (38) administered 30 g of alcohol to 2 male subjects and 20 g to 2 female subjects; the subjects worked for 1 hour, took alcohol plus drug, then worked for 2 more hours. Then followed a 30-minute rest, 30 minutes of work, 90 minutes for lunch, and 90 minutes of work. Methamphetamine (9 mg to males, 6 mg to females) not only counteracted the decrease in performance induced by alcohol but raised performance above control (no alcohol) levels. Caffeine (150 mg to males, 100 to females) prevented a fall in performance after alcohol but produced no enhancement.

A number of tasks requiring the subject to remain attentive has been investigated. Hollingworth (52) observed a slight increase in speed of typing and a considerable decrease in typing errors after a dose of 180 or 360 mg of caffeine. Hollingworth also observed an increased rate of naming colors on a card after caffeine in doses of 72 to 216 mg. Berdie (11) found no effect on a similar color-naming task of 15 mg of amphetamine given 2 hours before the test. Barmack and Seitz (10) found no effect of 10 mg of amphetamine on the number of letters

their subjects could reproduce after seeing a group of 8 flashed on a screen. Several investigators have examined the efficiency of crossing-out certain letters or digits randomly distributed among other letters or digits, and reported slight but variable stimulation by caffeine in one instance (52), and significantly faster performance after 600 to 900 mg of caffeine citrate in another (72). Lehmann and Csank (72), Adler *et al.* (1), and Tyler (109) also reported improved performance after amphetamine.

Dill *et al.* (25) gave subcutaneous injections of 20 mg of amphetamine to subjects working at a simulated altitude of 10,000 to 24,000 feet. This treatment affected neither arithmetic performance nor a simple coding task, but the decrease in oxygen concentration had no effect by itself, indicating that the task was insensitive.

Thus, on simple tasks such as arithmetic problems and cancellation, both caffeine and amphetamine can at times improve performance. Determination of whether this represents a true facilitation or merely an antidote to boredom requires that more demanding tasks be used.

A digit-symbol substitution test derived from the Wechsler Adult Intelligence scale was incorporated into experiments by Townsend and Mirsky (107) and Kornetsky *et al.* (65). The Townsend and Mirsky experiment demonstrated no effect of 5 or 15 mg of *d*-amphetamine. Kornetsky *et al.*, who used sleep-deprived subjects, found that the loss in performance produced by 68 hours without sleep disappeared after the administration of 15 mg of *d*-amphetamine. In a more complex task—solving war staff problems—Somerville (103) found that the administration of amphetamine, even to subjects without sleep for 42 hours, did not facilitate performance.

Andrews (3) could find no effect of amphetamine (10 mg) on the solution of syllogisms. Hecht and Sargent (48) could find no effect on rate of solving anagrams. In both experiments, however, the interval between drug administration and testing (30 minutes and 60 minutes, respectively) may not have been long enough to detect effects produced at the time of peak drug action. Two-move chess problems were used as the criterion by Holck (50); the problems and solutions were taken from a book. A dose of 200 mg of caffeine, subcutaneously, produced no demonstrable effect on performance of this task.

During the 1930's, several investigators (*e.g.*, 77, 78, 95) claimed that amphetamine could raise intelligence test scores. These studies suffer badly from the lack of adequate controls, *i.e.*, random allocation of subjects to treatments, placebo groups, double-blind designs, *etc.* The early promising results have not been duplicated by later work. Thus, Barmack (8) found no effect of 10 mg of amphetamine on the Otis Test scores of college students. Cutler *et al.* (22) gave mentally defective children 5 mg of amphetamine per day for 3 months and 7.5 mg per day for the next 3 months. This double-blind study could demonstrate no effect of the medication on intelligence test scores. Another group gave mentally defective subjects 5 mg of amphetamine per day for the first week, 10 mg per day for the second, and 15 mg per day for the third and fourth weeks of their well-controlled study (79). The authors concluded, "It is apparent that

treatment with amphetamine does not increase intelligence, learning capacity, speed and accuracy of voluntary attention, fluency, or memory in mental defectives."

Cattell (17) found no effect of 200 or 400 mg caffeine citrate, either on an intelligence scale or on a test of factual knowledge. However, his subjects began the tests only 20 minutes after taking the drug, which was probably insufficient time for peak effects to appear. Flory and Gilbert (29) evaluated the effects of 300 mg of caffeine citrate and 15 mg of amphetamine sulfate on rate of reading, reading comprehension, and on a vocabulary and analogies test. No effects of the drug were apparent.

The evidence at hand, therefore, indicates that neither amphetamine nor caffeine possesses properties which lead to improved intellectual performance except, perhaps, when normal performance has been degraded by fatigue or boredom.

VII. JUDGMENT AND MOOD

Is there a psychological price that must be paid for the use of drugs that enhance performance? Only two aspects of this question will be discussed here: 1) Do drugs that enhance performance impair judgment? 2) Do such drugs produce changes in mood that could be considered deleterious?

A. Effects on judgment

If one conceives of judgment as the appropriateness of behavior to the environment, then a drug could impair judgment in two ways. One would be directly related to certain subjective effects of the drug, *e.g.*, inflated estimates of one's ability to continue physically fatiguing work or to stay awake while driving an automobile. Such an impairment in judgment would be due to a lack of correlation between the subjective and performance-modifying effects of a drug. A second type of impairment would be more difficult to relate directly to known effects of the drug, for instance, reckless driving, poor tactics in a military operation, and so on. Neither of these facets of judgment has received much study. What little published work exists, moreover, treats only the amphetamines.

Davis (23) made two statements about judgment in his summary article on the British experience with stimulants during World War II. First, he stated that "the subject who has taken amphetamine usually judges the effects more favorably than the experimenter." Second, he claimed that amphetamine may induce a person to persist in inappropriate behavior. Winfield (111), however, in studying RAF bomber crews on combat missions, reported that no evidence of recklessness or lack of judgment was apparent after 10 or 15 mg of amphetamine. Somerville (103) was another investigator who could find no effect of amphetamine on judgment in a military situation. Officers working on an arduous 72-hour program of staff duty exercises were not permitted to sleep during the first 42 hours. Two opportunities for sleep were given during the last 30 hours, one for 4 hours, and one for 6 hours. The men worked in groups of 10 or 11, and their

performance was judged by general staff officers familiar with the tasks. Three treatments were chosen: no drug (N = 23), placebo (N = 25), and active drug (N = 25). Subjects in the active drug group received 20 mg of amphetamine in divided doses between the 32nd and 42nd hours. This dose was repeated between the 56th and 66th hours. Identically appearing inert capsules were given to those in the placebo group at the same times. The supervisory officers were asked to pay particular attention to any evidence of deficiencies in judgment. (They were kept unaware of which subjects received the active drug.) Little evidence was seen of the drug's producing any adverse effect upon judgment.

The ability of a subject to predict his future performance on the Air Force SAM Pursuit Confusion Task was not affected by 5 mg of *d*-amphetamine, according to Hauty and Payne (42). The subject estimated how long he would be able to keep a stylus in contact with a moving target on his next trial. Since he performed the task while viewing the apparatus in a mirror, interference effects from habitual perceptual-motor behavior produced a high degree of uncertainty about his performance. He thus had to depend heavily on immediate past performance in making his estimate for the next trial. Performance was measured 1 hour after the administration of the drug. Neither the algebraic nor the absolute differences between estimated and actually attained proficiency proved to be statistically significant, nor did the drug produce any changes in performance.

Smith and Beecher (102), whose work on amphetamine and athletic performance was discussed earlier, also gathered data on how accurately their subjects estimated their speed. A dose of 14 mg amphetamine/70 kg given 2 or 3 hours before the trial led subjects swimming alone and unfatigued to give lower estimates than those they gave under control conditions. This occurred in the face of an actual improvement in speed. Amphetamine did not produce this result, however, when the subjects were tired or when they competed with two other swimmers. The authors were unwilling to conclude that the drug impaired judgment. That it probably did affect time-estimation is indicated by the results of two other investigators. Goldstone *et al.* (36) presented each of their subjects with a series of audible signals of varying duration and then had them judge whether each signal was greater or less than 1 second in duration. The judgments were made 30 and 60 minutes after taking capsules containing either 15 mg of *d*-amphetamine, 200 mg of secobarbital, or a placebo. Compared to the placebo, *d*-amphetamine led subjects to overestimate the length of the signal, with the effect greater after 60 minutes than after 30 minutes. Using a totally different technique, Dews and Morse (24) showed a similar effect in a situation that required subjects to space successive key-presses a certain minimum length of time apart in order to win money. The subjects tended to press slightly earlier when they worked 30 minutes after an oral dose of 5 mg of *d*-amphetamine.

A mild time-distortion is therefore likely to occur with the amphetamines. It is also clear that much more work is needed before we can answer the question of whether the amphetamines have more general effects upon the judiciousness of decisions.

B. Effects on mood

The dominant subjective effect of the amphetamines, a general increase in feelings of alertness and well being, was reported in the early uncontrolled studies. Nathanson (80) was the first to study the effects of an amphetamine in a controlled fashion. He gave either 1) 10 mg of amphetamine before breakfast and 10 mg before lunch, or 2) 20 mg of amphetamine before lunch, or 3) placebos. Each subject (all were members of a hospital staff) was given a scaled questionnaire with instructions to open it and answer the questions in the evening. In reporting the results, Nathanson pooled his drug data, presumably because he found little difference between the two dosage schedules. A total of 55 subjects were in the drug groups, 25 in the placebo group. The differences were dramatic. Thirty-seven of the subjects that received the drug, but only one of those receiving the placebo, reported euphoria and feelings of exhilaration. Thirty of those on drug reported an increase in energy and in the desire and capacity for work; again, only one individual in the placebo group responded in this way. Comparable figures for an increase in talkativeness were 31 and 1; for feeling less fatigued from the day's work, 34 and 1; for feeling an increase in mental activity and efficiency, 23 and 0. On the negative side, 10 in the drug-group felt more fatigued from the day's work *versus* 2 in the placebo-group. And, while no one in the placebo-group reported any insomnia that night, 17 of those who had received the active drug reported either mild or moderate difficulty in going to sleep. Surprisingly little has been added to our knowledge of the effects of the amphetamines on verbal descriptions of mood since this early questionnaire study.

Bahnsen *et al.* (4) also studied the actions of amphetamine in an everyday context. They gave the drug to adult men and women, partially adjusting dose to weight by giving 20 mg to the men and only 10 mg to the women. There were 100 subjects in the experimental group and 95 in the group given placebos. The drug was taken in the morning of a normal working day and the subjects filled out extensive questionnaires before drug administration, at about 5:00 P.M. that afternoon, and the next morning. Apart from the usual lessening of fatigue, they reported "a considerable increase in the desire for work" and "the feeling that it is relatively easy to perform a task." Other items showing large differences between the drug and placebo groups were reports of general well being, good humor, exhilaration, talkativeness, restlessness, excitement, and anxiety. All these sensations were increased by the drug. A study by Jacobsen and Wollstein (59) in which they gave 15 mg of amphetamine to young men yielded similar findings.

One early and frequently cited attempt to study the effects of amphetamine on a wide variety of tasks as well as on mood was marred by an inadequate randomization procedure (14, 15, 108). In discussing one aspect of the results, Turner and Carl wrote: "It will be noted that the 'starting points' for the four groups differ from each other to a considerable degree and particularly that the 'P' (Placebo) group registered well above the others at first administration. It is quite likely that this condition is explicable in part through the fact that about one-third of the subjects in the 'P' group were under 21 years of age, who had

been so classified in order to avoid the necessity for securing the parental authority to administer Benzedrine; and, as indicated previously that there is a rather clear tendency for young subjects to register higher scores" (108, p. 166). Since the age-bias therefore was considerable, the results of the study are impossible to interpret.

As part of an intensive investigation of boredom and fatigue, Barmack (6) studied the effects of amphetamine on several mood variables. The subjects filled out rating scales after each of eight 15-minute work periods, during which they added pairs of 6-place numbers. The drug (or placebo) was given between 15 and 40 minutes before the first of the work periods. Each of 36 subjects served twice, once after 10 mg of amphetamine and once after the placebo, with both possible orders of administration used equally often The results were clearcut: with only a placebo, subjects reported that they became bored, inattentive, fatigued, sleepy, relaxed, and irritated as time wore on. The drug, while it did not abolish these decrements completely, reduced them considerably. Approximately the same result occurred when a slightly higher dose of amphetamine, 15 mg, was used with another task, the Poffenberger pursuitmeter (7). In this second experiment the drug was given one-half hour before the beginning of eight 15-minute work periods. The effects of 60 mg of ephedrine hydrochloride were equivocal.

Barmack (9) also studied the effects of 120 mg of caffeine on several mood variables as part of his investigation of its effects on addition. The drug slowed the development of boredom, fatigue, inattentiveness, and sleepiness, but had little effect either on the number of additions attempted or upon subjective ratings made early in the work session. The caffeine was given either 1, 2, or 3 hours before the start of the session in order to discover whether the effect was merely a function of time of administration. It was not; the subjective ratings seemed to be affected only if the subject had been working for a while, irrespective of the time since drug administration. A somewhat similar result was shown for *d*-amphetamine by Pearson and Byars (90). They found that 5 mg of the drug affected only slightly the subjective ratings of fatigue made by men who simply sat around filling out checklists every one and one-half hours for 6 hours, starting at 9:30 A.M.

Ten mg of amphetamine reduced the fatigue of men working at a simulated altitude of 16,000 feet, but did not bring their ratings back to the level found under ground control conditions, according to Seitz and Barmack (98). The judgments of fatigue were made 2 hours after the subjects had taken either the drug or a placebo. Adler *et al.* (1) found that 10 mg of amphetamine, 10 mg of *d*-amphetamine, 5 mg of methamphetamine, 420 mg of caffeine sodium benzoate, and 5 mg of amphetamine plus 225 mg of caffeine sodium benzoate all reduced the number of subjective symptoms reported at simulated altitudes of 15,000 and 18,000 feet.

Amelioration of the feelings of fatigue developed by prolonged work is the most fully documented subjective effect of the amphetamines. During the second World War, investigators in many countries tried to find out how well

these drugs worked and how much had to be paid for their use in the way of adverse side-effects. Work done in the United States has been reported by Seashore and Ivy (97) and Tyler (109); in Canada, by Somerville (103); in Great Britain, by Cuthbertson and Knox (21), Davis (23), and Winfield (111).

As discussed previously (pp. 3–4), Seashore and Ivy (97) conducted a series of eight field trials in which various stimulants were tested on infantry soldiers, truck drivers, and tank drivers. The one experiment reported in detail concerned the effects of 10 mg of amphetamine, 5 mg of methamphetamine, and 450 mg of caffeine sodium benzoate. The drugs and a placebo were given orally. An all-day hike was followed by continuous guard duty from 6:00 P.M. to 3:00 or 4:30 A.M. The experimenters then administered the 1.5 hour-long test battery, results from which have been described earlier. The data of interest to us here come from a check list given to the subjects some time during this period. One dose of the drugs was given at 6:00 P.M. and another at midnight. Each of 16 subjects received a different agent on each of four weekly occasions. The order was balanced so that no drug was favored. All three stimulants increased the estimates of how long the subjects thought they could have continued on guard duty. Amphetamine produced the fewest negative subjective symptoms. These included excessive sleepiness, vertigo, difficulty in concentrating, slowness in reasoning, nervousness, inward tension, restlessness, indifference, exhaustion, and tremor. Caffeine produced the next lowest incidence of subjective symptoms. Methamphetamine in the dose employed differed only slightly from the placebo. A comparison of positive subjective symptoms also showed amphetamine to be first, caffeine second, methamphetamine third, and the placebo last. The positive symptoms were: no sleepiness, talkativeness, excitement, and exhilaration. The authors also said that they "were often able, by the more buoyant attitude and behavior exhibited, to distinguish subjects receiving Benzedrine from those receiving the other two stimulants." The rest of the experiments in the series generally confirmed the results of this one, although the effects were less pronounced.

Tyler (109) carried out a series of studies on the performance of large groups of men forced to remain awake for as long as 112 hours. In one 72-hour experiment, he gave 10 mg of amphetamine every 8 to 12 hours, starting at either the 36th or 48th hour of wakefulness. The drug decreased the difficulty subjects had in remaining awake. Drug-induced mood changes were nearly absent, perhaps because no very systematic attempts were made to observe them, although Tyler attributed the lack of mood changes to the high motivational level of his subjects. One interesting finding was that more amphetamine subjects than placebo subjects were willing to volunteer for a 35-mile forced march with full pack on their third night of sleep deprivation. This may be indicative of deficiency in judgment, because they already had made two night marches totalling 69 miles and, according to Tyler, a third was out of the question since their feet were sore and blistered.

A rather surprising lack of effectiveness of amphetamine was reported by Somerville (103). Experiments were carried out on Canadian soldiers engaged in

obstacle course running, night and day marches, and prolonged staff duty exercises. Doses of amphetamine varied from 15 mg 1 hour before the end of the fatiguing activity to 40 mg given in divided doses over a 72-hour period. The differences that did occur were in the direction of a slight alerting effect of the drug. The only statistically significant difference was the number of subjects in the staff duty exercise experiment who thought the drug helped keep them awake (21 of 25 in the amphetamine group, 7 of 25 in the placebo group). Flory and Gilbert (29) and Cranston *et al.* (20) also failed to find significant subjective effects after amphetamine, as did Goldstein *et al.* (35). The failure of Kenyon and Pronko (61) to find mood changes (as measured by adjective sorting) after 10 mg of *d*-amphetamine could have been due to the shortness of the period between drug administration and testing (45 minutes). Another possibility that could account for the lack of subjective effects in the above studies is the insensitivity or inappropriateness of the measuring device. Dureman and Scholander (26) compared two kinds of subjective rating scales for sensitivity to drug effects. One contained 28 expressions related to non-specific activation; the subject checked, for each item, "more than," "alike," or "less than." The other consisted of three graphic rating scales representing "clearness of mind," "wakefulness-alertness," and "power of concentration"; the subjects rated these in three graded levels. On the check list, 10 mg of amphetamine produced a substantial increase in score, the greatest effect occurring at 180 minutes after administration of the drug. Many more subjects reported no effect on the rating scale after exactly the same dose. Subjective effects are just as dependent upon techniques of measurement as are performance effects, although it is rare to find investigators who act on this principle.

In contrast to some of the above results, Davis (23), in his short summary of the British World War II studies, reported an abundance of subjective effects with amphetamine. He stated that in subjects kept awake all night a dose of 15 mg of amphetamine may cause them to feel "more alert than usual and ready for anything. They may become mildly elated and confident, without doubts and anxieties and the discomfort of the tension usually experienced before starting a test. On the other hand, tests which without amphetamine are regarded as boring may engage their interest and become absorbing. They may feel able to concentrate on them without the usual diversion of their attention by extraneous events. Attention may seem to be narrowed and focused upon the task in hand."

Davis also believed that 15 mg of amphetamine often leads to impulsive and inappropriate activity, irritability, and restlessness, when the task given to the subject constrains his activity or calls for separate responses that must be made to separate stimuli at about the same time. This author reported great variability in response to the drug and saw more mood change and overt behavior change than anyone else. Thus: "Individual differences are large and unpredictable, and the effects differ in the same individual from occasion to occasion. Sometimes individuals become excited, restless, and garrulous. Some become irritable and quarrelsome, others emotionally labile. . . . Irresponsible behaviour, euphoria and airy, dreamy or drunk feelings may also result from a 15 mg dose." Unfortunately, no quantitative data were given, nor were the methods of observa-

tion. Davis did not state whether these kinds of data were systematically gathered or whether his statements were the result of casual observations. One cannot judge the validity of his conclusions under these circumstances.

Cuthbertson and Knox (21) kept soldiers without sleep for 24 hours, then marched them 18 miles. A dose of 15 mg of methamphetamine was given just before the march. The 55 subjects were divided into three squads. All the members of one squad were given the drug, all the members of the second were given a placebo, and the third squad was made up of approximately equal numbers of drug and placebo subjects. By arranging matters in this way, rather than randomly assigning both treatments within each squad, the authors made possible an interesting reanalysis of their data that shows how the euphoriant effect of the drug was magnified by placing like-treated subjects together. In the all-drug group, 10 of 18 subjects reported having experienced euphoria during the march, whereas in the all-placebo group, only 1 of 17 reported euphoria; the difference was statistically significant (chi-square = 8.38, dF = 1, $P < .01$). But the incidences of euphoria in the group with approximately half the subjects on drug and half on placebo, were 1 out of 8 for the former and 0 out of 10 for the latter. Thus, it seems that the euphorigenic effect was much more pronounced when everyone in the group received the drug. This social facilitation effect has been studied, with other drugs, by Wendt and the Nowlises at the University of Rochester (83).

Lasagna *et al.* (68) examined how amphetamine and several other drugs affected the reports of mood of two widely disparate groups, normal volunteers (predominantly university students) and former narcotic addicts. (Data from a third group of old, chronically ill patients were not very satisfactory, since many of the patients were unable to cooperate fully.) The subjects filled out a standard questionnaire 30 minutes, 1 hour, and 2 hours after receiving the drug. Normal subjects received a subcutaneous injection of either saline placebo, 20 mg of amphetamine, 2 or 4 mg of heroin hydrochloride, or 8 or 15 mg of morphine phosphate. Sodium pentobarbital, in doses of 50 and 100 mg, was given intravenously. The former addicts were given either the saline placebo, 20 or 30 mg of amphetamine, 4 or 6 mg heroin hydrochloride, or 15 or 22.5 mg of morphine phosphate. All doses were per 70 kg of body weight. The subjects did not know which drugs they were getting.

Amphetamine surpassed all the other drugs in its ability to create a pleasant state in normal subjects. It achieved the highest score on a euphoria index compiled from the questionnaire items. This contrasted sharply with the dysphoria produced by the two opiates. It also was the most popular drug when subjects were asked to rate them all on a "most pleasant" to "most unpleasant" scale at the end of the experiment. Fifteen of the 20 subjects put amphetamine on the "pleasant" side of the midpoint; the rest called it "unpleasant." Only five of them called the placebo pleasant; one called it unpleasant, and the rest found it neutral. Amphetamine was much more popular than any of the other drugs, with pentobarbital second, and the opiates liked by few subjects and heartily disliked by most.

Amphetamine also greatly improved the mood of the former addicts according

to the results of the euphoria index rating. In fact, on this score, which reflected only the first 2 hours after drug administration, it produced as pleasant a reaction as did the opiates. (As might be expected, because of their special history, the former addicts contrasted greatly with the normal subjects in enjoying the opiates.) Amphetamine did not maintain its popularity on the ratings made at the end of the entire experiment, apparently because the former addicts were unhappy with the insomnia and other side-effects that sometimes resulted. Thirty subjects thought it pleasant; eight either called it neutral or had no reaction, and nine called it unpleasant. (The 20- and 30-mg dose data were combined because they did not differ significantly.) In contrast, one subject called the saline placebo pleasant, 25 called it neutral, and 4 called it unpleasant.

Another approach to the study of the effects of drugs on mood was taken by the group at the University of Rochester (83). They have used a variety of agents in an extensive exploration of changes in affect in subjects working together in four-man groups. The primary tool has been a list consisting of 100 to 200 adjectives on which the subject is asked to check those that apply to him. Amphetamine alone was used in some of the early work of this group. A dose of 10 mg of amphetamine typically increased the checking of such words as "businesslike, talkative, capable, enterprising, independent, nervous, jittery," and it decreased the checking of such words as "lazy, languid, nonchalant."

Smith and Beecher (101) also showed that amphetamine produces a positive mood report. A variety of doses—usually 14 mg/70 kg, sometimes 7 or 21 mg/70 kg—influenced the way their college runners, swimmers, and weight throwers checked a list of adjectives quite similar to the one used by the Rochester group. The authors summarize their findings in this way:

"The increased feeling of mental and physical activation was the most definite amphetamine effect . . . there was more checking of such words as active, vigorous, and energetic on amphetamine than on placebo days; less checking of sluggish, weak, drowsy, and tired; more checking of alert and clearheaded; less checking of mentally slow; more checking of efficient, ambitious, industrious, and effective; and more checking of excited, on edge, anticipative, tense, jittery, and restless on amphetamine than on placebo days. The second most definite amphetamine effect was increased elation. There was more checking of elated, exhilarated, happy, cheerful, and overjoyed and less checking of depressed and moody on amphetamine days than on placebo days. A third positive effect was increased boldness. The subjects checked bold, boastful, cocky, self-confident, playful, and domineering more, and insecure less, on amphetamine days than on placebo days. The fourth definite effect was greater friendliness, as indicated by more checking of friendly, talkative, good-natured, obliging, and trustful and less checking of grouchy, unsociable, and sarcastic" (p. 1509).

Ritter *et al.* (92) took another approach to the problem of uncovering possible deleterious effects of a drug. They reasoned that "the psychologic stress imposed upon individual organisms by the adjustments involved in getting along together toward some common goal may constitute a sufficient condition for the exposure of latent unwanted effects of *d*-amphetamine." The task they chose was the

USAF SAM Two-Hand Coordination Test (76), modified so that each subject had control of one handle but only one subject could see the turntable and the stylus that the team attempted to keep in contact with the moving disc. At the end of the task each subject was asked to make ratings of his attitudes toward the task itself, his own performance, and his partner's performance. A dose of 5 mg of *d*-amphetamine, given 1 hour before the start of the group session, significantly improved the subjects' attitudes toward the task. Attitudes toward one's own and his partner's performance were also more positive after *d*-amphetamine but the changes were smaller and not statistically significant. Thus, the drug did not cause adverse effects; on the contrary, what effects it did have were all positive.

A 5-mg dose of *d*-amphetamine produced a more favorable attitude toward the prolonged tracking task used by Payne and Hauty (84) than any other pharmacological treatment. The attitude score associated with a caffeine derivative was higher than that with the placebo, but far less than that with *d*-amphetamine. By analyzing the relation between attitude scores and performance, the authors showed further that the effects of the drugs on performance could not be ascribed to favorable attitudes (*cf.* 69).

Several investigators have combined a barbiturate with an amphetamine in the hope that the combination would prove an equally good stimulant but without the negative effects occasionally reported. Unfortunately, no direct extensive comparisons have been made with the amphetamine alone. There is some evidence that the combination produces somewhat more euphoric and outgoing behavior (83). Laties (69) studied the effects of 10 mg of amphetamine plus 100 mg of secobarbital on the mood of college students who worked in four-man groups when deprived of sleep for about 36 hours and when not deprived of sleep. The Nowlis Adjective Check List was used several times before and during the experiment. About 2.5 hours after oral drug administration, whether sleep-deprived or not, subjects reported more friendliness, more involvement with their tasks, more social initiative, and more elation than they did after placebos. This picture is strikingly similar to that reported by Smith and Beecher (101) for amphetamine alone: more friendliness, more activation, more boldness, and more elation.

Lanzetta *et al.* (67) used a slightly smaller dose of the same combination, 7.5 mg of amphetamine and 75 mg of secobarbital, in a study of three-man groups working on a task that the subjects believed was being used as an evaluation procedure for their military reserve unit. Under the influence of the drugs, subjects checked more adjectives on a check-list that connoted "a relaxed, positively affective, cooperative, phenomenal state," while they checked fewer adjectives that connoted "an anxious, obstructive state."

About the only reliable information on the subjective effects of caffeine apart from the work by Barmack (9) comes from Hollingworth's monograph (52). Hollingworth gauged these effects by inspecting the diaries his subjects were asked to keep. Although, because of the hard work involved in the tests, many subjective complaints were noted on control days, a greater incidence was noted

on days during which caffeine, in doses of 240 mg and more, had been given. The most prominent symptoms included nervousness, feverishness, headache, irritability, and disturbed sleep.

VIII. CONCLUDING REMARKS

The foregoing evidence indicates that a very wide range of behavior (with the notable exception of intellectual tasks) can be enhanced by caffeine and the amphetamines—all the way from putting the shot to monitoring a clock face. Moreover, the superiority of the amphetamines over caffeine is unquestionable.

Two questions are implicit in these conclusions. 1) How do these drugs enhance performance; can they actually produce superior performance or do they merely restore performance degraded by fatigue, boredom, and so on? 2) What is the "cost" of obtaining this enhancement; is it great enough to prohibit the practical use of these agents, particularly the amphetamines?

Davis (23), who studied amphetamine, and Barmack (6, 7, 9), who studied both amphetamine and caffeine, are among those who claim that these agents produce their effects not by an increase in capacity, but by making people more interested in the task, or as Barmack (6) states, because their effect is to allay the development of unfavorable attitudes toward the task. Davis' evidence is unconvincing, but Barmack supports his argument with data. One of his most cogent findings in this regard was the lack of effect of caffeine on subjects who had just started working *versus* its effects on subjects who had been working for 2 hours. Also, he found with amphetamine that the greatest differences between active drug and placebo (on adding 6-place numbers) occurred toward the end of the work-period and paralleled reports of boredom, irritation, and inattention by the control subjects.

To support this contention we also have the data of Kornetsky *et al.* (65), who found that *d*-amphetamine affected performance only in subjects suffering from prolonged sleep-loss. Furthermore, Hauty and Payne (44) showed that in highly motivated subjects, *d*-amphetamine did not produce the same superiority in performance relative to placebo that it produced in subjects who typically showed early decrement of performance. Finally, Mackworth's (73) results showed only a restoration by amphetamine to normal performance on a vigilance task, and no enhancement. Moreover, a number of experiments have failed to show any effects at all in non-fatigued subjects.

Opposing these data and their interpretation, however, are arrayed data at least equally convincing. Among the most cogent are those of Smith and Beecher (100) on athletic performance. Smith and Beecher (experiment 5) found that amphetamine significantly decreased the time to swim an event, even in college swimmers so highly motivated that they often exceeded, with placebo, the best times that they had made in intercollegiate competition. Smith and Beecher also found (experiment 1) that the effects of amphetamine were more apparent in rested than in fatigued subjects. Furthermore, shot-putting and weight-throwing displayed the greatest proportionate modifications by the drug. It is difficult to see how fatigue, lack of interest, or boredom could be significant factors in such an acute expenditure of effort.

Some of the findings of the Randolph Field investigators also support the notion that drugs can have effects above and beyond a mere restoration of previously impaired performance. Their data frequently showed a sizable increase in proficiency after the administration of *d*-amphetamine relative to the highest score achieved during the pre-drug period (41, 45, 84, 88). Even though this increased proficiency may not be statistically significant when the studies are evaluated individually, the consistency of this finding suggests that the effect is a real one. Another consistent finding of these investigators is a difference in the kind of improvement produced by psychological and pharmacological variables; only drugs were able to forestall the decline in proficiency with time. Other investigators who have found performance improved beyond the control level in non-fatigued subjects include Adler *et al.* (1), Lehmann and Csank (72), and Eysenck *et al.* (27). The results of Adler *et al.* are particularly noteworthy. In their experiment, *d*-amphetamine not only restored to normal some kinds of performance degraded by exposure to a simulated altitude of 18,000 feet, but brought other kinds to levels better than that seen at ground level. And the two studies by Hauty *et al.* (45, 46) conclusively demonstrated on a monitoring task that *d*-amphetamine could prevent proficiency decline even with the added challenge of inadequate oxygen. Payne *et al.* (87) also offered data that contradict Barmack's finding (6) that the difference between amphetamine and placebo is exaggerated toward the end of a long work period. They found an equivalent effect of a particular dose no matter when during the period it was given.

Actually, neither Barmack's data nor those of the other investigators who hold similar views can justify their position that drugs that enhance performance do so only by inducing favorable attitudes. It is quite likely that drugs such as the amphetamines produce at least two independent effects—performance changes and attitude changes. The fact that these parallel one another does not necessarily confirm a deterministic relationship; nor does it make it unnecessary to show that attitudinal variance can account for performance variance. Indeed, in the only investigations to take this crucial step, the subjective data argued against the hypothesis that the amphetamines improve performance merely by inducing favorable attitudes toward the task. Payne and Hauty (84) found that favorable dispositions toward tasks which arose from special instructions were not reflected in performance. They also demonstrated that very significant variation in drug response occurred independently of attitude variation. Pearson (89) obtained similar results; he could demonstrate no more than a very slight correlation between fatigue ratings and performance decrement. One can point to even more dramatic disparities; for instance, although alcohol may increase confidence in the ability to drive an automobile, we have every reason to doubt that this parallels an increased ability to perform the task.

The associated claim that only performance degraded by fatigue and similar states can be helped by drugs also suffers from a logical defect. Obviously, if one is working just below a task ceiling the effect of an agent is more difficult to detect than if performance is well below that ceiling. One should not confuse ease of detection with mechanism of action.

There are strong indications, therefore, that the amphetamines, and perhaps

caffeine as well, can do more than merely restore performance degraded by factors such as muscular fatigue, sleep deprivation, and boredom.

The evidence for answering the second question—is there a cost to the enhancement obtained?—is mostly negative. Both from the standpoint of physiological and psychological cost, amphetamines and caffeine are rather benign agents. Except for reports of insomnia, the subjective effects of the amphetamines in normal doses are usually favorable. Moreover, no one has ever presented convincing evidence that they impair judgment. Caffeine seems somewhat less benign. Hollingworth's (52) subjects, after doses of about 240 mg and above, reported such symptoms as nervousness, feverishness, irritability, headache, and disturbed sleep. Caffeine also produces a significant increase in tremor. At dose levels that clearly enhance performance, the amphetamines seem not only more effective than caffeine, but less costly in terms of side-effects.

These statements refer only to the acute effects of caffeine and the amphetamines. Are additional costs incurred when these drugs are taken chronically to enhance performance? Do they lead to addiction? There appears to be no experimental evidence from which to answer this question. The clinical reports show that occasional individuals, usually persons with neurotic or psychotic symptoms, habitually take extremely high doses. However, there is no evidence of physical dependence; abrupt cessation of the drug produces hardly any effect, apart, perhaps, from a transient somnolence (56a, 62a, 112). Caffeine also does not produce physical dependence. Withdrawal in habitual coffee drinkers seems mainly to lead to an increased incidence of headaches for a day or two (66). Some degree of tolerance develops to both; neither is addicting in the sense that narcotics are. In view of the wide use enjoyed by these drugs, the incidence even of habituation, so far as one can tell from the literature, is quite low. [The sociological aspects of the use of these drugs, particularly the amphetamines, are discussed by Leake (70)].

A final word is in order about our present state of knowledge. Strikingly few attempts have been made to determine the basic parameters of drug action and performance. Such work is essential if we are ever to develop broad principles. What little we have learned has had to be inferred from work rarely designed to yield unambiguous answers. The question of whether the effects of amphetamine are dependent upon the amount of degradation of performance existing at the time the drug is taken has been discussed above. Few other broad questions have stimulated even this amount of work. A few examples should suffice to make the point. (a) We suspect that amphetamine affects coordination and tracking tasks differentially depending upon the complexity of the task used (Sect. III). But task complexity has rarely been made the subject of explicit study. (b) Work with lower animals suggests that the effects of the amphetamines on response rate are in part dependent upon the rate at which the subject is responding when given the drug (23a); almost no systematic work on human performance has been done (24). (c) There is a hint that the mood changes due to any drug depend upon the environment of the subject; more specifically, there is some evidence that amphetamine leads to more profound changes when the subject

is surrounded by others also on the drug. Little has been done to determine the variables operating here (21, 83). (d) We have no information on whether tolerance develops to the effects of these drugs on performance as it does, say, to the anorexigenic effects of the amphetamines. It is clear that the notion of drug-behavior interaction, which is proving so important in behavioral pharmacology (98a), should be applied more frequently to work on the human level.

REFERENCES

1. Adler, H. F., Burkhardt, W. L., Ivy, A. C. and Atkinson, A. J.: Effect of various drugs on psychomotor performance at ground level and simulated altitudes of 18,000 feet in a low pressure chamber. J. Aviat. Med. 21: 221-236, 1950.
2. Alles, G. A. and Feigen, G. A.: The influence of benzedrine on work decrement and patellar reflex. Amer. J. Physiol. 136: 392-400, 1942.
3. Andrews, T. G.: The effect of benzedrine sulfate on syllogistic reasoning. J. exp. Psychol. 25: 423-431, 1940.
4. Bahnsen, P., Jacobsen, E. and Thesleff, H.: The subjective effect of beta-phenylisopropylaminesulfate on normal adults. Acta med. scand. 97: 89-131, 1938.
5. Balloch, J. C., LaSaine, T. A. and Robinson, J. M.: An experimental study of the effects of dexedrine (d-amphetamine sulfate) upon motor and mental performance and some factors in mood. J. Tenn. Acad. Sci. 27: 296-303, 1952.
6. Barmack, J. E.: The effect of benzedrine sulfate (benzyl methyl carbinamine) upon the report of boredom and other factors. J. Psychol. 5: 125-133, 1938.
7. Barmack, J. E.: Studies on the psychophysiology of boredom. 1. The effect of 15 mgs. of benzedrine sulfate and 60 mgs. of ephedrine hydrochloride on blood pressure, report of boredom and other factors. J. exp. Psychol. 25: 494-505, 1939.
8. Barmack, J. E.: The effect of 10 mgs. of benzedrine sulfate on the Otis test scores of college students. Amer. J. Psychiat. 97: 163-166, 1940.
9. Barmack, J. E.: The time of administration and some effects of 2 grs. of alkaloid caffeine. J. exp. Psychol. 27: 690-698, 1940.
10. Barmack, J. E. and Seitz, C. P.: The effect of 10 mgs. of benzedrine sulfate on the span of attention for letters. J. gen. Psychol. 23: 195-196, 1940.
11. Bendie, R. F.: Effect of benzedrine sulfate on blocking in color naming. J. exp. Psychol. 27: 326-332, 1940.
12. Bøje, O.: Doping: a study of the means employed to raise the level of performance in sport. Bull. World Hlth Org. 8: 439-469, 1939.
13. Bujas, Z. and Petz, B.: Utjecaj fenamina na ekonomičnost statičnog rada. Arh. hig. rada. 6: 205-208, 1955.
14. Carl, G. P. and Turner, W. D.: The effects of benzedrine sulfate (amphetamine sulfate) on performance in a comprehensive psychometric examination. J. Psychol. 8: 165-216, 1939.
15. Carl, G. P. and Turner, W. D.: A further report on benzedrine sulfate (amphetamine sulfate): psycho-physical effects, and supplementary results from a fifth experimental group. J. gen. Psychol. 22: 185-191, 1940.
16. Carpenter, J. A.: The effect of caffeine and alcohol on simple visual reaction time. J. comp. Physiol. Psychol. 52: 491-496, 1959.
17. Cattell, R. B.: The effects of alcohol and caffeine on intelligence. Brit. J. med. Psychol. 10: 20-33, 1930.
18. Cheney, R. H.: Comparative effect of caffeine per se and a caffeine beverage (coffee) upon the reaction time in normal young adults. J. Pharmacol. 53: 304-313, 1935.
19. Cheney, R. H.: Reaction time behavior after caffeine and coffee consumption. J. exp. Psychol. 19: 357-360, 1936.
19a. Cochran, W. G., Smith, G. M. and Beecher, H. K.: Amphetamine sulfate and performance. A reply. J. Amer. med. Ass. 177: 347-349, 1961.
20. Cranston, R. E., Zubin, J. and Landis, C.: The effect of small doses of thonzylamine, dexedrine and phenobarbital on test performance and self-rating of subjective states. J. Psychol. 33: 209-215, 1952.
21. Cuthbertson, D. P. and Knox, J. A. C.: The effects of analeptics on the fatigued subject. J. Physiol. 106: 49-58, 1947.
22. Cutler, M., Little, J. W. and Strauss, A. A.: The effect of benzedrine on mentally deficient children. Amer. J. ment. Defic. 45: 59-65, 1940.
23. Davis, D. R.: Psychomotor effects of analeptics and their relation to fatigue phenomena in air crew. Brit. med. Bull. 5: 43-45, 1947.
23a. Dews, P. B.: Analysis of effects of psychopharmacological agents in behavioral terms. Fed. Proc. 17: 1024-1030, 1958.
24. Dews, P. B. and Morse, W. H.: Some observations on an operant in human subjects and its modification by dextro amphetamine. J. exp. Anal. Behav. 1: 359-364, 1958.
25. Dill, D. B., Benson, O. O., Forbes, W. H., Hall, F. G., Michelsen, J. and Knehr, C. A.: Benzedrine sulfate and acute anoxia. I. Respiratory effects; II. Effects on cardiovascular system; III. Mental work. J. Aviat. Med. 11: 181-186, 1940.
26. Dureman, I. and Scholander, T.: Subjective estimation of drug-induced changes in activation level. Scand. J. Psychol. 1: 36-40, 1960.

27. EYSENCK, H. J., CASEY, S. AND TROUTON, D. S.: Drugs and personality: II. The effect of stimulant and depressant drugs on continuous work. J. ment. Sci. 103: 646–649, 1957.
28. EYSENCK, H. J. AND EASTERBROOK, J. A.: Drugs and personality: VI. The effects of stimulant and depressant drugs upon body sway (static ataxia). J. ment. Sci. 106: 831–834, 1960.
29. FLORY, C. D. AND GILBERT, J.: The effects of benzedrine sulfate and caffeine citrate on the efficiency of college students. J. appl. Psychol. 27: 121–134, 1943.
30. FOLTZ, E. E., IVY, A. C. AND BARBORKA, C. J.: The use of double work periods in the study of fatigue and the influence of caffeine on recovery. Amer. J. Physiol. 136: 79–86, 1942.
31. FOLTZ, E. E., IVY, A. C. AND BARBORKA, C. J.: The influence of amphetamine (benzedrine) sulfate, d-desoxyephedrine hydrochloride (pervitin) and caffeine upon work output and recovery when rapidly exhausting work is done by trained subjects. J. Lab. clin. Med. 28: 603–605, 1943.
32. FOLTZ, E. E., SCHIFFRIN, M. J. AND IVY, A. C.: The influence of amphetamine (benzedrine) sulfate and caffeine on the performance of rapidly exhausting work by untrained subjects. J. Lab. clin. Med. 28: 601–603, 1943.
33. FRANKS, C. M. AND TROUTON, D.: Effects of amobarbital sodium and dexamphetamine sulfate on the conditioning of the eyeblink response. J. comp. Physiol. Psychol. 51: 220–222, 1958.
34. GILLILAND, A. R. AND NELSON, D.: The effects of coffee on certain mental and physiological functions. J. gen. Psychol. 21: 339–348, 1939.
35. GOLDSTEIN, A., SEARLE, B. W. AND SCHIMKE, R. T.: Effects of secobarbital and of *d*-amphetamine on psychomotor performance of normal subjects. J. Pharmacol. 130: 55–58, 1960.
36. GOLDSTONE, S., BOARDMAN, W. K. AND LHAMON, W. T.: Effect of quinal barbitone, dextro-amphetamine, and placebo on apparent time. Brit. J. Psychol. 49: 324–328, 1958.
37. GOLLA, F. L., BLACKBURN, J. M. AND GRAHAM, S.: A comparison between some of the effects of isomyn (benzedrine) and of methylisomyn. J. ment. Sci. 86: 48–59, 1940.
38. GRAF, O.: Increase of efficiency by means of pharmaceutics (stimulants). In: German Aviation Medicine, World War II, vol. 2, pp. 1080–1103. U. S. Govt. Printing Office, Washington, D. C., 1950.
39. HALDI, J. AND WYNN, W.: Action of drugs on efficiency of swimmers. Res. Quart. 17: 90–101, 1946.
40. HAUTY, G. T.: The effects of drugs upon the components of hand steadiness. Rpt. No. 5, Proj. 21-1601-0004, USAF School of Aviation Medicine, Texas, 1954.
41. HAUTY, G. T. AND PAYNE, R. B.: Mitigation of work decrement. J. exp. Psychol. 49: 60–67, 1955.
42. HAUTY, G. T. AND PAYNE, R. B.: Effects of *dextro*-amphetamine upon judgment. J. Pharmacol. 120: 33–37, 1957.
43. HAUTY, G. T. AND PAYNE, R. B.: Effects of analeptic and depressant drugs upon psychological behavior. Amer. J. publ. Hlth 48: 571–577, 1958.
44. HAUTY, G. T. AND PAYNE, R. B.: Fatigue, confinement, and proficiency decrement. In: Vistas in Astronautics, ed. by M. Alpern and M. Stern, pp. 304–309. Pergamon Press, New York, 1958.
45. HAUTY, G. T., PAYNE, R. B. AND BAUER, R. O.: Effects of normal air and *dextro*-amphetamine upon work decrement induced by oxygen impoverishment and fatigue. J. Pharmacol. 119: 385–389, 1957.
46. HAUTY, G. T., PAYNE, R. B. AND BAUER, R. O.: Physiological costs incurred by dextro-amphetamine. J. comp. Physiol. Psychol. 50: 647–651, 1957.
47. HAWK, P. B.: A study of the physiological and psychological reactions of the human organism to coffee drinking. Amer. J. Physiol. 90: 380–381, 1929.
48. HECHT, R. AND SARGENT, S. S.: Effects of benzedrine sulfate on performance in two tests of high mental functions. J. exp. Psychol. 28: 528–533, 1941.
49. HELLEBRANDT, F. A. AND KARPOVICH, P. V. Fitness, fatigue and recuperation: survey of methods used for improving the physical performance of man. War Med., Chicago 1: 745–768, 1941.
50. HOLCK, H. G. O.: Effect of caffeine upon chess problem solving. J. comp. Physiol. Psychol. 15: 301–311, 1933.
51. HOLLAND, J. G.: Human vigilance. Science 128: 61–67, 1958.
52. HOLLINGWORTH, H. L.: The influence of caffeine on mental and motor efficiency. Arch. Psychol., N. Y. 3: no. 22, 1–166, 1912.
53. HORST, K., BUXTON, R. E. AND ROBINSON, W. D.: The effect of the habitual use of coffee or decaffeinated coffee upon blood pressure and certain motor reactions of normal young men. J. Pharmacol. 52: 322–337, 1934.
54. HORST, K., ROBINSON, W. D., JENKINS, W. L. AND BAO, D. L.: The effect of caffeine, coffee and decaffeinated coffee upon blood pressure, pulse rate and certain motor reactions of normal young men. J. Pharmacol. 52: 307–321, 1934.
55. HORST, K. AND JENKINS, W. L.: The effect of caffeine, coffee and decaffeinated coffee upon blood pressure, pulse rate and simple reaction time of men of various ages. J. Pharmacol. 53: 385–400, 1935.
56. HULL, C. L.: The influence of caffeine and other factors on certain phenomena of rote learning. J. gen. Psychol. 13: 249–274, 1935.
56a. ISBELL, H. AND WHITE, W. M.: Clinical characteristics of addictions. Amer. J. Med. 14: 558–565, 1953.
57. IVY, A. C. AND GOETZL, F. R.: D-Desoxyephedrine: a review. War Med., Chicago 3: 60–77, 1943.
58. IVY, A. C. AND KRASNO, L. R.: Amphetamine (benzedrine) sulfate: a review of its pharmacology. War Med., Chicago 1: 15–42, 1941.
59. JACOBSEN, E. AND WOLLSTEIN, A.: Studies on the subjective effects of the cephalotropic amines in men. I. Beta-phenylisopropylamine sulfate. Acta med. scand. 100: 159–187, 1939.
60. KARPOVICH, P. V.: Effect of amphetamine sulfate on athletic performance. J. Amer. med. Ass. 170: 558–561, 1959.
61. KENYON, G. Y. AND PRONKO, N. H.: Dexedrine (*d*-amphetamine sulfate) and laboratory induced anxiety. Psychol. Rep. 7: 415–433, 1960.

62. KLEEMEIER, L. B. AND KLEEMEIER, R. W.: Effects of benzedrine (amphetamine) on psychomotor performance. Amer. J. Psychol. 60: 89–100, 1947.

62a. KNAPP, P. H.: Amphetamine and addiction. J. nerv. ment. Dis. 115: 406–432, 1952.

63. KNOEFEL, P. K.: The influence of phenisopropyl amine and phenisopropyl methyl amine on work output. Fed. Proc. 2: 83, 1943.

64. KORNETSKY, C.: Effects of meprobamate, phenobarbital and *dextro*-amphetamine on reaction time and learning in man. J. Pharmacol. 123: 216–219, 1958.

65. KORNETSKY, C., MIRSKY, A. F., KESSLER, E. K. AND DORFF, J. E.: The effects of *dextro*-amphetamine on behavioral deficits produced by sleep loss in humans. J. Pharmacol. 127: 46–50, 1959.

66. LANDIS, C.: Physiological and psychological effects of the use of coffee. In: Problems of Addiction and Habituation, ed. by P. H. Hoch and J. Zubin, pp. 37–48. Grune and Stratton, New York, 1958.

67. LANZETTA, J. T., WENDT, G. R., LANGHAM, P. AND HAEFNER, D.: The effects of an "anxiety-reducing" medication on group behavior under threat. J. abnorm. (soc.) Psychol. 52: 103–108, 1956.

68. LASAGNA, L., VON FELSINGER, J. M. AND BEECHER, H. K.: Drug-induced mood changes in man. I. Observations on healthy subjects, chronically ill patients, and post addicts. J. Amer. med. Ass. 157: 1006–1020, 1955.

69. LATIES, V. G.: Modification of affect, social behavior and performance by sleep deprivation and drugs. J. psychiat. Res. 1: 19–25, 1961.

70. LEAKE, C. D.: The Amphetamines, Charles C Thomas, Springfield, Ill., 1958.

71. LEHMANN, G., STRAUB, H. AND SZAKALL, A.: Pervitin als leistungssteigerndes Mittel. Arbeitsphysiologie 10: 680–691, 1939.

72. LEHMANN, H. E. AND CRANK, J.: Differential screening of phrenotropic agents in man. J. clin. Psychopath. 18: 222–235, 1957.

73. MACKWORTH, N. H.: Researches on the Measurement of Human Performance. Medical Research Council, Special Rpt. Series no. 268, H. M. Stationery Off., London, 1950.

74. MCNAMARA, W. J. AND MILLER, R. E.: The effect of benzedrine sulfate on mental work. Psychol. Rec. 1: 78–84, 1937.

75. MAYER, J. AND BULLEN, B.: Nutrition and athletic performance. Physiol. Rev. 40: 369–397, 1960.

76. MELTON, A. W., ed.: Apparatus tests, AAF Aviation Psychology Program Research Reports, Rpt. No. 4, U. S. Govt. Printing Office, Washington, 1947.

77. MOLITCH, M. AND ECCLES, A. K.: The effect of benzedrine sulfate on the intelligence scores of children. Amer. J. Psychiat. 94: 587–590, 1937.

78. MOLITCH, M. AND SULLIVAN, J. P.: The effect of benzedrine sulfate on children taking the New Stanford Achievement Tests. Amer. J. Orthopsychiat. 7: 519–522, 1937.

79. MORRIS, J. V., MACGILLIVRAY, R. C. AND MATHIESON, C. M.: The results of the experimental administration of amphetamine sulfate in oligophrenia. J. ment. Sci. 101: 131–140, 1955.

80. NATHANSON, M. H.: The central action of beta-aminopropylbenzene (benzedrine). Clinical observations. J. Amer. med. Ass. 108: 528–531, 1937.

81. NEWMAN, H. W.: Effect of amphetamine sulfate on performance of normal and fatigued subjects. J. Pharmacol. 89: 106–110, 1947.

82. NEWMAN, H. W. AND NEWMAN, E. J.: Failure of dexedrine and caffeine as practical antagonists of the depressant effect of ethyl alcohol. Quart. J. Stud. Alc. 17: 406–410, 1956.

83. NOWLIS, V. AND NOWLIS, H. H.: The description and analysis of mood. Ann. N. Y. Acad. Sci. 65: 345–355, 1956.

84. PAYNE, R. B. AND HAUTY, G. T.: The effects of experimentally induced attitudes upon task proficiency. J. exp. Psychol. 47: 267–273, 1954.

85. PAYNE, R. B. AND HAUTY, G. T.: Factors affecting the endurance of psychomotor skill. J. Aviat. Med. 25: 382–389, 1955.

86. PAYNE, R. B. AND HAUTY, G. T.: Effect of psychological feed-back upon work decrement. J. exp. Psychol. 50: 343–351, 1955.

87. PAYNE, R. B., HAUTY, G. T. AND MOORE, E. W.: Restoration of tracking proficiency as a function of amount and delay of analeptic medication. J. comp. Physiol. Psychol. 50: 146–149, 1957.

88. PAYNE, R. B. AND MOORE, E. W.: The effects of some analeptic and depressant drugs upon tracking behavior. J. Pharmacol. 115: 480–484, 1955.

89. PEARSON, R. G.: Task proficiency and feelings of fatigue. School of Aviation Medicine, USAF, Rpt. 57-77, April, 1957.

90. PEARSON, R. G. AND BYARS, G. E.: The development and validation of a checklist for measuring subjective fatigue. School of Aviation Medicine, USAF, Rpt. 56-115, 1956.

90a. PIERSON, W. R.: Amphetamine sulfate and performance. A critique. J. Amer. med. Ass. 177: 345–347, 1961.

91. RASCH, P. J., PIERSON, W. R. AND BRUBAKER, M. L.: The effect of amphetamine sulfate and meprobamate on reaction time and movement time. Int. Z. angew. Physiol. 18: 280–284, 1960.

92. RITTER, R. M., PAYNE, R. B. AND HAUTY, G. T.: The effects of drugs and controlled progress feedback upon two-member team behavior. School of Aviation Medicine, USAF, Rpt. 57-58, 1957.

93. RIVERS, W. H. R. AND WEBBER, H. N.: The action of caffeine on the capacity for muscular work. J. Physiol. 36: 33–47, 1907.

94. RUTENFRANZ, J. AND JANSEN, G.: Über die Kompensation von Alcoholwirkungen durch Caffein und Pervitin bei einer psychomotorischen Leistung. Int. Z. angew. Physiol. 18: 62–81, 1959.

95. SARGANT, W. AND BLACKBURN, J. M.: The effect of benzedrine on intelligence scores. Lancet 231: 1385–1387, 1936.

96. SCHILLING, W.: The effect of caffeine and acetanilid on simple reaction time. Psychol. Rev. 28: 72–79, 1921.

97. SEASHORE, R. H. AND IVY, A. C.: Effects of analeptic drugs in relieving fatigue. Psychol. Monogr. 67: no. 15, 1–16, 1953.

98. Seitz, C. P. and Barmack, J. E.: The effects of 10 mgs. of benzedrine sulfate and low oxygen tension on the span of attention for letters, and other factors. J. Psychol. 10: 241–247, 1940.
98a. Sidman, M.: Drug-behavior interaction. Ann. N. Y. Acad. Sci. 65: 289–302, 1956.
99. Simonson, E. and Enzer, N.: The effect of amphetamine (benzedrine) sulfate on the state of motor centers. J. exp. Psychol. 29: 517–523, 1941.
100. Smith, G. M. and Beecher, H. K.: Amphetamine sulfate and athletic performance. I. Objective effects. J. Amer. med. Ass. 170: 542, 1959.
101. Smith, G. M. and Beecher, H. K.: Amphetamine, secobarbital, and athletic performance. II. Subjective evaluations of performance, mood, and physical states. J. Amer. med. Ass. 172: 1502–1514, 1960.
102. Smith, G. M. and Beecher, H. K.: Amphetamine, secobarbital, and athletic performance. III. Quantitative effects on judgment. J. Amer. med. Ass. 172: 1623–1629, 1960.
103. Somerville, W.: The effect of benzedrine on mental or physical fatigue in soldiers. Canad. med. Ass. J. 55: 470–476, 1946.
104. Switzer, S. A.: The influence of caffeine upon "inhibition of delay." J. comp. Psychol. 19: 155–175, 1935.
105. Switzer, S. A.: The effect of caffeine on experimental extinction of conditioned reactions. J. gen. Psychol. 12: 78–90, 1935.
106. Thornton, G. R., Holck, H. G. O. and Smith, E. L.: The effects of benzedrine and caffeine upon performance in certain psychomotor tasks. J. abnorm. (soc.) Psychol. 34: 96–113, 1939.
107. Townsend, A. M. and Mirsky, A. F.: A comparison of the effects of meprobamate, phenobarbital and d-amphetamine on two psychological tests. J. nerv. ment. Dis. 130: 212–216, 1960.
108. Turner, W. D. and Carl, G. P.: Temporary changes in affect and attitude following ingestion of various amounts of benzedrine sulfate (amphetamine sulfate). J. Psychol. 8: 415–453, 1939.
109. Tyler, D. B.: The effect of amphetamine sulfate and some barbiturates on the fatigue produced by prolonged wakefulness. Amer. J. Physiol. 150: 253–262, 1947.
110. von Felsinger, J. M., Lasagna, L. and Beecher, H. K.: Drug-induced mood changes in man. 3. Personality and reactions to drugs. J. Amer. med. Ass. 157: 1113–1119, 1955.
111. Winfield, R.: Use of benzedrine to overcome fatigue on operational flights in bomber command. Great Britain: Flying Personnel Research Committee, Rpt. No. 498 (no date).
112. Zondek, L.: Amphetamine abuse and its relation to other drug addictions. Psychiat. neurol., Basel 135: 337–346, 1958.

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Accidental Caffeine Poisoning in a Dog

The following case report demonstrates the danger of pets gaining access, accidentally, to drugs for human use.

A 4-month-old mongrel dog, weighing about 15 lb., swallowed some tablets* containing approximately 50 gr. of caffeine and, in two hours, it was in a state of collapse—its body in a semitetanic state. Its temperature was 104 F., its pulse was rapid and weak, the mucous membranes were pale, and the pupils were dilated, with a sluggish reflex.

The dog was given 3 cc. of pentobarbital sodium intraperitoneally, but it died before further measures could be taken.

There was no warning of poison or toxicity on the box. If this tablet is toxic for a dog, it probably is just as toxic for a child under similar circumstances.—*Fred J. Wigderson, V.M.D., Hackensack, N.J.*

*The author expresses his appreciation to Dr. John H. Collins of the Food and Drug Administration for the analysis of this product.

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The Methyl Xanthines—A New Class of Goitrogens

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ABSTRACT. Rats chronically fed methyl xanthines in a low iodine diet developed goiters. The potency of the compounds tested was in the order: theophylline = aminophylline > caffeine > theobromine. Goitrogenesis was reduced on high iodine diets and absent in hypophysectomized rats. Theophylline caused marked, synergistic enhancement of the goitrogenic effect of small doses of propylthiouracil. Aminophylline and theophylline lowered the PBI but at theophylline concentrations prevailing in the sera little antithyroid activity could be demonstrated in rat thyroid segments *in vitro*. It is concluded that the methyl xanthines are mild antithyroid and strongly goitrogenic compounds. The possibility that this dissociation between the antithyroid and goitrogenic response involves mediation by 3',5'-AMP is discussed. (*Endocrinology* 85: 410, 1969)

THE UNEXPLAINED rarity of iodide goiter despite the widespread use of iodides in chronic respiratory disorders and in certain diets (1) led us to search for possible cogoitrogens—*i.e.*, agents that would enhance the effect of a potential goitrogen such as excess iodide. Such a relationship apparently exists between iodide and certain sulfonamides. Thus, goitrogenesis is potentiated by iodide (2, 3), and iodide markedly increases the antithyroid potency of sulfadiazine as measured by organic iodine formation (4). Ingbar and co-workers (5) have recently shown a similar synergism between iodide and phenazone—a combination of subgoitrogenic doses of these agents led to goiter.

Since the methyl xanthines, and particularly theophylline, are commonly used in conjunction with iodides in medications, the combined effect of these agents was investigated on the rat thyroid gland. Although there was no cogoitrogenic effect with combined iodide and aminophylline feeding for three weeks, the methyl xanthines were found to be goitrogenic and, more importantly, they markedly enhanced the goitrogenicity of small doses of propylthiouracil.

Materials and Methods

Male Sprague-Dawley rats weighing 120 ± 10 g were obtained locally or from the Hormone Assay Laboratories, Chicago. Hypophysectomized rats were obtained from the Hormone Assay Laboratories, Chicago. Rats were fed a modified Remington low iodine diet *ad lib.* and given tap water. The diet consisted of 3 parts of regular Remington diet plus 1 part of vitamin-fortified low iodine diet, both obtained from Nutritional Biochemical Corp. The iodine content of this mixture was 0.033 μg/g by the chloric acid method (Boston Medical Laboratories).

The methyl xanthines (obtained from Mann and aminophylline (theophylline, ethylenediamine) (obtained from Sigma) were mixed with the diet in dry form. Because of the small concentrations used, propylthiouracil (PTU) was first mixed with the diet to yield a concentrated diet which was then used in the appropriate amounts to mix the final concentration. The diets, plus tap water, were given *ad lib.* for 21 days unless otherwise stated.

For measurement of the *in vitro* antithyroid activity of theophylline, rat thyroids were obtained from animals fed the low iodine diet. Each lobe was divided into 4–6 parts and one such part from 10–12 rats was used flask (~20 mg tissue/2.0 ml) which was incubated at 37 C for 150 min in Krebs-Ringer phosphate medium containing $1 \cdot 10^{-2}$M glucose and $1 \cdot 10^{-6}$M NaI labeled with $^{131}I^-$. At the end of incubation the pieces were blotted, weighed and placed in glass homogenizers in which the radioactivity was counted both before and after precipitation with trichloroacetic acid (TCA) as previously described (6). Incubations in rat serum (from experiments listed in Table 2) were carried out in 1.0 ml of serum to which 5 μl of 1.0M Tris pH 7.4 had been added, as well as enough NaI to yield a final concentration of $1 \cdot 10^{-6}$M. The procedure was otherwise identical.

Serum methyl xanthine levels were deter

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TABLE 1. Effect of aminophylline and excess iodide on thyroid size

	Thyroid weight mg/100 g ± SE
Control (7)	8.7 ± 0.89
Iodide (7)	5.8 ± 0.28
Aminophylline (8)	12.1 ± 0.74
Iodide + aminophylline (8)	6.5 ± 0.34

Iodide was mixed into the drinking water [100 μg I^- (as NaI) per ml]. The concentration of aminophylline was 0.1% in low iodine diet. Numbers in parentheses refer to the number of rats per group.

mined by a modification of the method of Schack and Waxler (7). One ml of serum pooled from 2 rats bled in the morning after *ad lib.* feeding) was extracted with 15 ml of a mixture of chloroform and 2-propanol, 20:1, in a separatory funnel. The organic phase was transferred into a graduated glass stoppered centrifuge tube. The serum was re-extracted with 10 ml of chloroform-propanol and the organic phases were combined and centrifuged briefly to separate any trapped aqueous phase. This was removed by suction. The surface was washed with 1 ml H_2O and the water was removed with enough chloroform-propanol to leave 21 ml of the organic solvent. The latter was extracted with 5.0 ml of 0.10N NaOH and centrifuged. Absorbancies of the aqueous phases were determined with control sera as blanks at 277 mμ for theophylline and 274 for theobromine. Concentrations were determined from standard curves similarly prepared and PBI values by the Boston Medical Laboratories.

Results

As demonstrated in Table 1, feeding of iodide reduced thyroid weight in both control and aminophylline fed rats ($p < 0.01$). This experience is like that usually seen with thiocarbamides (1), and provides evidence against any synergism between these two agents, at least for the three-week period of these experiments. Identical results were obtained when the iodide intake of the two iodide groups was increased 10-fold.

Also apparent from Table 1 is the fact that aminophylline was itself goitrogenic when fed in a low iodine diet for three weeks ($p < 0.01$). To rule out any possible effects of the ethylenediamine (present in aminophylline to increase the solubility of theophylline), the common methyl xanthines were compared for their goitrogenic effect. At a dietary concentration of 10 μmoles/g the order of increasing effectiveness was theobromine < caffeine < theophylline = aminophylline (Table 2). While methyl xanthine concentrations of the serum were comparable for the drugs used, the PBI was significantly lowered only in the two groups of rats receiving theophylline preparations. These rats also had by far the largest goiters. Nevertheless, caffeine produced thyroid enlargement in the absence of any decrease in the PBI (Table 2). Ethylenediamine alone had no effect at this dosage. It is of interest that 1,7-dimethylxanthine (paraxanthine) was at one time stated to have antithyroid properties (8, 9). However, this claim could not subsequently be confirmed by two other investigators (10, 11).

A dose-response curve for theophylline is depicted in Fig. 1. Since the largest dose caused body weight losses, we routinely chose a concentration of 10 μmoles/g of diet. With this level of drug the weight gain was nearly comparable to that of control rats (Table 2). However, to rule out effects of altered dietary intake, pair feed-

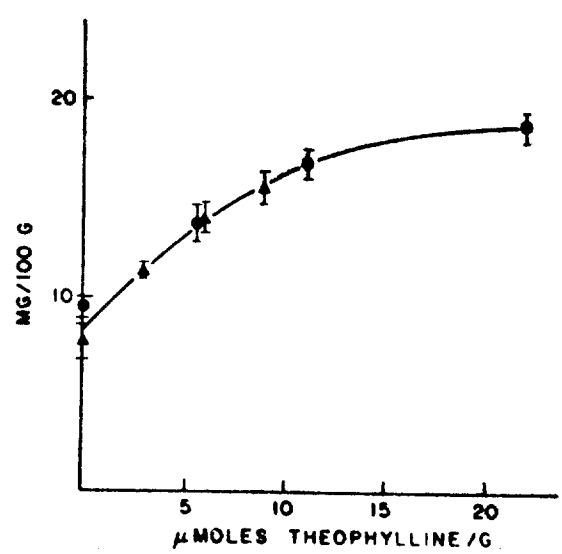

FIG. 1. Dose response curve for the effect of theophylline on thyroid size. All diets were fed for 3 weeks *ad lib.* Each point is the average of 7–8 rats. The SE is indicated. Triangles and circles are from separate experiments.

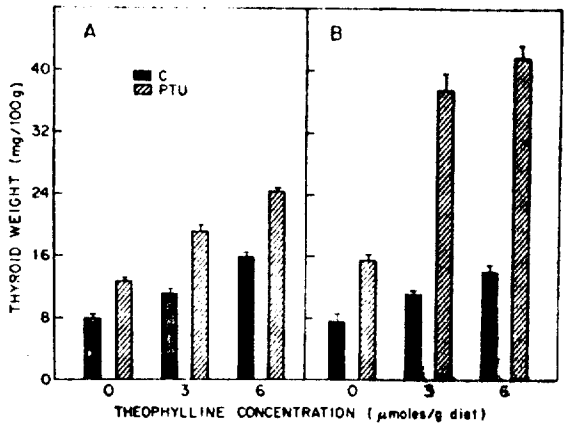

FIG. 2. Synergism of the goitrogenic effects of propylthiouracil and theophylline. C = control, PTU = propylthiouracil. All diets were fed for 3 weeks. In experiment A the PTU concentration was 0.01 μmole/g of low iodine diet; in B it was 0.018 μmole/g. Each bar represents the mean and SE of 7–8 rats in exp A and 6 rats in exp B. Mean PBI values of rats of exp A were 2.6, 2.5 and 1.5 μg/100 ml for the 0, 3 and 6 μmoles/g theophylline concentrations, respectively, and 1.8, 1.7 and 1.6 μg/100 ml for the equivalent groups receiving PTU as well.

ing experiments were carried out with theophylline. These show clearly that goitrogenicity is real and not a result of body weight changes (Table 3).

As with other goitrogens, an intact pituitary gland was necessary in order to obtain thyroid enlargement with theophylline (Table 3). While the hypophysectomized rats tolerated theophylline poorly even at a lowered dosage, no difference existed between control and theophylline-treated hypophysectomized rats. Moreover, the mean serum theophylline level at time of sacrifice was 0.26 mM, suggesting that there was adequate exposure to the drug. Although a low iodine diet more suitable for hypophysectomized rats than the Remington diet was used here, similar results were obtained with the standard low iodine diet. It should also be noted that thyroid glands receiving minimal thyrotropic stimulation such as occurs in rats fed a high iodine diet (Purina Lab Chow) show much less, but significant ($p < 0.01$), enlargement under otherwise similar conditions (Table 3).

Because of the well-known effect of the methyl xanthines on mammalian 3′,5′-AMP diesterase (12), it was important to distinguish between an antithyroid and a purely goitrogenic effect (enhancement of TSH stimulation). As shown in Table 4 only the largest concentration of theophylline ($1 \cdot 10^{-3}$M) caused any reduction (~20%) of thyroid iodide uptake without a change in the fraction present in TCA-insoluble form. In other experiments the effect was even less marked and beef thyroid slices showed no inhibition at $3 \cdot 10^{-3}$M. While the inhibitory concentrations are thus probably greater than attained *in vivo* under our conditions ($2–5 \cdot 10^{-4}$M), the values listed in Table 2 and for the rats used in Fig. 2 were obtained at sacrifice (in the morning) and may not represent maxima. On the other hand, the method used (7) will not distinguish certain metabolites such as monomethylxanthines (13). Sera of rats bearing goiters due to theophylline feeding showed a slight inhibition of iodine incorporation by rat thyroid segments *in vitro* (Table 4). This decrease was not statistically significant ($p > 0.05$).

Although the reduced PBI levels of rats

TABLE 2. Comparisons of the goitrogenicity of various methyl xanthines

Treatment	Initial g	Final g	Thyroid weight ±SE mg/100 g	Serum PBI μg/100 ml	Serum methyl xanthines mM
Control (10)	112	244	9.3 ±0.37	2.0	—
Theobromine (9) (3,7-Dimethylxanthine)	112	218	11.1 ±0.64	1.8	0.24–0.34
Caffeine (10) (1,3,7-Trimethylxanthine)	114	222	14.6 ±0.98	1.9	—
Aminophylline (9)	112	221	24.5 ±1.5	1.0	0.36–0.53
Theophylline (10) (1,3-Dimethylxanthine)	114	233	24.3 ±2.0	1.1	0.32–0.52

All drugs were fed at a concentration of 10 μmoles/g of low iodine diet for 24 days. The aminophylline concentration is per mole of theophylline contained therein. Numbers in parentheses refer to number rats/group. Serum PBI and methyl xanthine determination are means of 4–5 pools from 2 rats each.

TABLE 3. Effect of dietary conditions on goitrogenesis by theophylline

Condition	Treatment	Body weight (g)		Thyroid weight ±SE mg/100 g
		Initial	Final	
Pair-fed (Low iodine diet)	Control	100	193	7.6 ±0.41
	†Theophylline (5)	106	196	14.3 ±1.1
Hypophysectomized*	Control (6)	119	112	6.1 ±0.12
	‡Theophylline (8)	122	103	6.6 ±0.20
High iodine diet§	Control (10)	112	225	5.3 ±0.24
	†Theophylline (9)	116	208	7.5 ±0.43

All experiments lasted for 21 days.
* Started 1 week after hypophysectomy.
† 10 μmoles theophylline/g.
‡ 7.5 μmoles theophylline/g.
§ Purina Lab Chow.

receiving theophylline suggested an antithyroid effect, the goiters obtained on caffeine without PBI depression (Table 3) indicated the need for a search for goitrogenic properties other than those simply resulting from inhibition of hormone formation. Evidence was therefore sought for any synergism of theophylline and PTU in rats receiving small doses of PTU. From previous studies (14) and preliminary experiments, minimal dose combinations were established for the two goitrogens for differentiating between additive and synergistic effects. The results depicted in Fig. 2 point clearly to a synergistic effect when suitably small doses of PTU were employed. With the lower concentration of PTU the effect was less marked. Nevertheless, the expected sum of the individual thyroid weight responses would have been 16.0 mg/100 g for the 3 μmoles/g of theophylline dose and 20.7 mg/100 mg for the 6 μmoles/g theophylline dose. The actual mean weights found were 19.1 ±0.7 mg/100 g and 24.5 ±0.4 mg/100 g, respectively. With the larger (0.018 μmole PTU/g) concentrations, the synergism is striking for both concentrations of theophylline. In a single experiment (not shown) in which 0.033 μmole PTU/g was fed, the concomitant feeding of μmoles theophylline/g caused very little additional increase in thyroid weight over that taken from rats fed PTU alone.

Discussion

The distinction between antithyroid and goitrogenic effects is frequently obfuscated. The present findings make it clear, however, that these phenomena can and should be considered independently of each other in some circumstances. Thus, antithyroid activity is undiminished in animals which show little or no goitrogenic response (*e.g.*, after hypophysectomy). Conversely, goitrogenesis occurs with uninterrupted iodine metabolism after TSH treatment. In the present study both an antithyroid and a goitrogenic response occur. While antithyroid activity *in vitro* was difficult to show at prevailing serum theophylline concentrations (Table 4), the serum PBI values of goitrous rats were decreased in a number of groups (Table 2). We have not ruled out extrathyroidal causes for decreased PBI levels or an increase in the proportion of circulating hormone present as triiodothyronine.

The goitrogenic response to the methyl xanthines appears to predominate as shown by:

1. The questionable *in vitro* antithyroid effect at concentrations prevailing in sera of rats bearing goiters (Table 4).
2. Significant thyroid enlargement in the absence of lowered PBI values (caffeine, Table 2; low doses of theophylline, Fig. 2).
3. Marked synergism with quite small doses of propylthiouracil.

The facts 1) that the methyl xanthines are inhibitors of mammalian cyclic 3′,5′-nucleotide phosphodiesterase (12), including the thyroid enzyme, and 2) that a number of TSH effects proceed through mediation by cyclic 3′,5′-AMP (15, 16), suggest

TABLE 4. Effect of theophylline on iodine metabolism *in vitro*

Exp	Theophylline concentration mM	Total iodine incorporated pmoles/mg	Fraction TCA precipitable
A	0	37	0.54
	0.10	36	0.52
	0.20	36	0.53
	0.40	40	0.53
	0.70	39	0.57
	1.00	29	0.55
B	Control sera	13.9	0.73
	Theophylline sera	12.2	0.72

All values obtained from a pool of rat thyroids, divided so as to distribute equal portions from each gland to each flask. In experiment A, theophylline was added to Krebs-Ringer phosphate medium. All values are duplicates. In experiment B, thyroids were incubated in sera from the study of Table 2. Values are means of 6 flasks each.

that the goitrogenesis produced by the methyl xanthines and synergism of the PTU effect operate, at least in part, through the maintenance of a higher level of 3′,5′-AMP. It should be pointed out that we have no direct proof for this, and measurement of tissue levels of the nucleotide will be required. Confirmation comes from the fact that the order of potencies of the methyl xanthines, *i.e.*, theophylline > caffeine > theobromine, is like that reported for inhibition of the specific diesterase (12, 17). If the present interpretation is correct, other inhibitors of the diesterase should also be goitrogenic in low iodine diets. This is currently being studied. Whether or not the potentiation of the TSH effect by thiourylene compounds that is sometimes reported (14, 18) results from an action like that of the methyl xanthines, in addition to iodine depletion (18), remains to be determined.

Since the pituitary gland may utilize the 3′,5′-AMP mechanism, it is possible that the effect on thyroid size seen in these studies results from increased secretion of TSH. It has been shown, however, that, six hours after the injection of 7.5 mg of theophylline/100 g, the TSH concentration of rat blood is not changed (19). Studies of TSH levels after chronic theophylline treatment are in progress. Of interest is the fact that, although the rat adrenal contains an active adenyl cyclase, there was no enlargement of the adrenal glands in these rats (controls: 20.9 mg/100 g, 10 μmoles theophylline/g for 21 days: 20.6 mg/100 g). This may be related to the inhibition of protein synthesis (glycine-^{14}C incorporation) in the adrenal stated to result from theophylline treatment (20). It is evident that such an effect does not play a major role in the thyroid.

Addendum

Serum TSH levels were measured by Dr. R. W. Bates with the chick iodine depletion method, pooled sera from the experiment of Fig. 2A. The following values, in mU/100 ml, were obtained: Control: 7.5, PTU: 27.5, theophylline 3 and 6 μmoles/g: 11.5 and 25, resp., PTU + theophylline 3 μmoles/g; 22.5 PTU + theophylline 6 μmoles/g: 35. It is clear that significant increases in thyroid weight can be obtained *without* increases in circulating TSH levels. This supports that part of the effect of the methyl xanthines is due to an increase in efficiency in the thyroidal response to TSH.

References

1. Wolff, J., *Amer J Med* (July 1969).
2. McKenzie, C. G., *Endocrinology* **40**: 137, 194[illegible]
3. Brown, J., and D. H. Solomon, *Endocrinology* **63**: 473, 1958.
4. Milne, K., and M. A. Greer, *Endocrinology* **71**: 580, 1962.
5. Pasternak, D. P., E. L. Socolow, and S. H. Ingbar, *Endocrinology* **84**: 769, 1969.
6. Wolff, J., and J. E. Rall, *Endocrinology* **76**: 949, 1965.
7. Schack, J. A., and S. H. Waxler, *J Pharmacol Exp Ther* **97**: 283, 1949.
8. Carter, G. S., F. G. Mann, J. Harley-Mason, and G. N. Jenkins, *Nature (London)* **151**: [illegible] 1943.
9. Carter, G. S., and G. N. Jenkins, *Nature (London)* **154**: 639, 1944.
10. Williams, R. H., and J. B. Peters, *J Clin Endocr* **5**: 217, 1945.
11. Barker, S. B., *Endocrinology* **37**: 484, 1945.
12. Butcher, R. W., and E. W. Sutherland, *J Biol Chem* **237**: 1244, 1962.
13. Cornish, H. H., and A. A. Christman, *J Biol Chem* **228**: 315, 1957.
14. Alexander, W. D., and J. Wolff, *Endocrinology* **78**: 581, 1966.
15. Pastan, I., and V. Macchia, *J Biol Chem* **242**: 5757, 1967.
16. Gilman, A. B., and T. W. Rall, *J Biol Chem* **243**: 5867, 5872, 1968.
17. Butcher, R. W., and E. W. Sutherland, *Pharmacologist* **1**: 63, 1959.
18. Bray, G. A., *J Clin Invest* **47**: 1640, 1968.
19. Bastomsky, C. H., and J. M. McKenzie, *Endocrinology* **83**: 309, 1968.
20. Halkirston, I. D. K., M. Feinstein, and [illegible] Hechter, *Proc Soc Exp Biol Med* **122**: 896, 1[illegible]

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COFFEE AND BLADDER CANCER

SIR,—An epidemiological study by Dr. Philip Cole [1] suggests an association between coffee drinking and bladder-cancer risk. Fraumeni and his colleagues [2] and Dunham and associates,[3] upon recomputation of their data using the statistical methods employed by Dr. Cole, also found an association in Negro women between coffee drinking and bladder cancer. However, a lack of dose/response relationship and other inconsistencies were noted by both groups, which led Fraumeni and his colleagues to the conclusion that the association was probably non-causal. We submit our observations on bladder pathology in rats, which are part of a study undertaken to define the chronic toxic and oncogenic potential of instant coffee.

Instant-coffee solids were administered for 2 years to Sprague-Dawley male and female rats, starting as 21-day-old weanlings. The instant coffee was incorporated into Wayne Laboratory Chow at a concentration of 5·0%. The caffeine content of the instant coffee was 3·6%, thus providing a dietary concentration of 0·18% caffeine. The instant-coffee level was chosen on the basis of preliminary studies undertaken to define the maximum dose accepted by the rat without obvious food refusal. On a weight basis, the rats consumed the equivalent of a man drinking 85 cups of strong coffee daily (150 ml. cups, each cup containing 2·2 g. instant-coffee solids and 79 mg. caffeine, or a total of 6·7 g. caffeine). 144 males and 144 females were used as test animals. A basic-diet control group contained 41 males and 41 females.

Necropsies and histopathological examinations were conducted in all animals which died during the study and in survivors killed after 2 years. The bladders of 94 males and 99 females in the test group and 29 males and 29 females in the control group were examined. None of the urinary bladders of animals examined showed abnormalities related to the coffee diet. Only coincidental bladder changes were noted, and in no case was a tumour found. Hyperplasia of the bladder epithelium was not seen, and only 1 rat, a control, had bladder stones. Persistent hyperplasia and bladder calculi have been implicated as predisposing factors in bladder-tumour induction.[4] Thus, no instant-coffee-related bladder tumours were noted in rats which had been fed diets containing as much as 5% instant-coffee solids over the major portion of the lifespan at a dosage level equivalent to the consumption, by a man, of 85 cups per day.

We must conclude that the association between coffee consumption and bladder cancer suggested by Dr. Cole and Dr. Fraumeni is not supported by the results obtained from long-term feeding experiments in rats.

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1. Cole, P. *Lancet*, 1971, i, 1335.
2. Fraumeni, J. F., Scotto, J., Dunham, L. J. *ibid.* 1971, ii, 1204.
3. Dunham, L. J., Rabson, A. S., Stewart, H. L., Frank, A. S., Young, J. L., Jr. *J. natn. Cancer Inst.* 1971, 41, 683.
4. British Industrial Biological Research Association (B.I.B.R.A.). Counting "Lumps" in the Rodent Bladder; vol. 10, no. 7, p. 317, 1971.

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THE EFFECT OF COFFEE ON THE FAT METABOLISM

by

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The effect of various pretreated types of pure coffee on the fat metabolism was examined in 44 patients with liver disease and 41 healthy subjects, and the results were compared with those obtained with caffein-free coffee. Mobilization of the free fatty acids from the deposited fat revealed a direct dependency upon the absolute caffein content of the coffee mixture, whether untreated or treated. On the other hand, the caffein-free coffee causes no stress in the fat metabolism. In the case of persons with liver disease, the intake of caffein or caffein-containing beverages should be avoided.

Besides the long known intolerability reactions, such as heartburn, stomachaches, spasms in the gastro-intestinal tract, restlessness, heavy beating of the heart and disturbed sleep, the consumption of coffee also leads to a direct affectation of the fat metabolism. Belle et al. (2) were first to observe that caffein-containing coffee leads to an increase in the free fatty acids in the blood. According to more recent studies, the increase in the free fatty acids in the plasma, resulting from the effect of caffein, should be given a pathogenetic significance for various diseases. Thus, in patients with angina pectoris, a significant increase in lipoproteins and total cholesterin was observed after protracted coffee intake; it is argued that the reason for this increase is a separation of free fatty acids from the deposited fat. (4) We ourselves were able to determine that in persons with liver disease, as opposed to healthy persons, caffein-containing coffee leads to a significantly more intense and longer-lasting increase in the free fatty acids (12). This effect is dependent upon the caffein content. After administration of caffein-free coffee, no alterations in the fat metabolism could be recognized.

In our further research, we were especially interested in the question of whether variously pretreated coffee mixtures influence the effect of pure coffee on the fat metabolism. Therefore, we compared several types of caffein-containing coffees, such as Columbia coffee, commercially steamed according to the method of Lendrich, with coffee washed with carbon dioxide according to the Bach method, and with decaffeinated coffee produced by the HAG method.

Observations and Methodology

1. Selection of patients

For the oral administration of caffein-containing or caffein-free coffee, a total of 85 test subjects were selected. Among these were 44 patients with liver disease, and 41 healthy subjects; the subjects were divided into 8 groups.

2. Experiments with Columbia coffee

Group I included 11 patients with liver disease, and Group II was composed of 10 healthy subjects. According to the analytical examinations, the amount of caffein in a 250 ml infusion of 20 g ground coffee amounted to 225 mg.

3. Experiments with steamed coffee

Group III was composed of 13 patients with liver disease, and Group IV of ten healthy subjects. The analytically determined caffein amount was 193 mg in an infusion of 20 g coffee.

4. Experiments with coffee pretreated with CO_2

Group V consisted of 10 patients with liver disease, and Group Vi of 9 healthy persons. 201 mg caffein were contained in an infusion of 20 g coffee.

5. Experiments with caffein-free coffee

Group VII consisted of 12 patients with liver disease, and Group VII of 10 healthy persons. The caffein content was 9 mg in 20 g coffee.

6. Preparation of the coffee infusion

300 ml boiling water were poured over 20 g ground coffee. After 7 minutes, the coffee was strained off and drunk by the subjects without any further ingredients being added. The amount of liquid of the coffee infusion drunk amounted to 250 ml.

7. Preparation of the subjects

Before commencement of the experiment, we determined in all the subjects the blood fat value (free fatty acids, esterified fatty acids and total cholesterol), serum lability tests (velocity of blood corpuscle decrease, takata, thymol, bilirubin and electrophoresis), liver function tests (galactose and bromothaleine tests), and serum enzyme activity (SGPT, SGOT and LDH). After about a 12-hour fast, blood was taken from each subject through the venules, to be used as a standard of reference. Finally, 250 ml of the corresponding coffee infusion were given to the subjects as a test drink, and the alterations in the blood fat level were observed for 360 minutes. Blood samples were taken after 30, 60, 120, 180, 240 and 360 minutes. We determined the total cholesterol and esterified fats according to a method described earlier (6), and the free fatty acids according to the method of Dole et al. (3).

Results

In the case of healthy persons, after oral intake of caffein-containing coffe, a significant increase in the free fatty acids in the plasma can be observed. Decaffeinated coffee leads, on the other hand, to no significant alterations in the free fatty acids in the plasma.

When we used caffein-containing Columbia coffee, the plasma free fatty acid concentration increased on the average from 0.41 to 0.89 μval/ml after three hours in healthy persons (figure 1). After 5 hours, it dropped back to the initial value. In the case of the patients with liver disease, the average value of the free fatty acids in the plasma was, at 1.08 μval/ml, considerably above the control value of the healthy persons. After oral administration of Columbia coffee, we could recognize an increase to 1.65 μval/ml.

Steamed coffee produced in healthy persons an increase in the free fatty acids from 0.32 to 0.71 μval/ml (figure 2), and in patients with liver disease from 0.97 to 1.39 μval/ml.

After coffee pretreated with carbon dioxide, the free fatty acids in the plasma of the healthy persons rose from 0.37 to 0.82 μval/ml (figure 3), and in the plasma of the liver diseased patients from 0.99 to 1.58 μval/ml.

Caffein-free coffee led to no significant increase in the free fatty acids in either the healthy persons (figure 4) or the patients with liver disease.

Discussion

Our studies again verify that in the case of patients with liver disease, as opposed to healthy subjects, an increased fast level of free fatty acids in the plasma can be determined, and that after oral intake of caffein-containing coffee, there is a significantly more intense and longer-lasting rise in the free fatty acids. A comparison of variously pretreated types of coffee and decaffeinated coffee enables us to recognize clearly that the increase in the free fatty acids in the plasma is directly dependent upon the caffein content. In accordance with its lower caffein content, steamed coffee leads to a less marked increase in the free fatty acids in the serum than does, for example, Columbia coffee or CO_2-treated coffee. Decaffeinated coffee, on the other hand, manifests no significant alterations in the serum free fatty acid level.

A special significance can be attributed to the metabolism of the free fatty acids insofar as about 60-80% of the total calorie requirements in the organism are covered by this form of energy. Even if the higher initial value of free fatty acids in the plasma of patients with liver disease is in no way pathognostic for liver diseases, this finding should nonetheless be considered appropriately. Normally, the liver can take up up to 50% of the free fatty acids separated from the deposited fat by enzymatic splitting of the triglycerides. The greater part of the free fatty acids bound to albumin are re-esterified to triglycerides and phosphatides and given off to the blood in the form of β-lipoproteins or reduced over acetyl coenzyme A to CO_2 and H_2O. This narrow relation of fat metabolism and liver function allows us to suppose that in the case of restricted liver function, the uptake

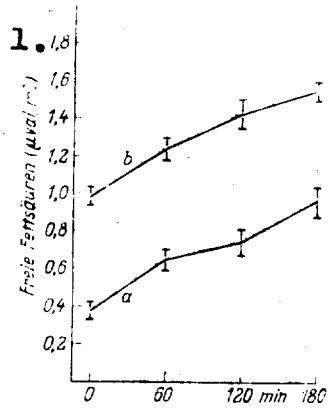
and use of free fatty acids, as well as the total fat metabolism, are reduced in the dmamaged liver of patients with liver disease. This assumption is supported by recent studies, which uniformly show that in the case of patients with liver disease, in an average of 60% of the cases, increased blood fat values can be determined as a manifestation of an accompanying fat metabolism disorder (10, 11). Other authors, in the meantime, have arrived at similar results (1, 7, 9).

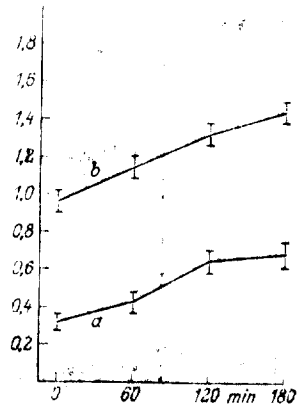
An increased separation of the deposited fat (5), as well as a disturbed intermediary carbohydrate metabolism in patients with liver disease have in the meantime also been discussed as the cause for the increased level of free fatty acids in the plasma (8). Most probably, however, the raised fast level of the free fatty acids in the case of liver parenchyma damage can be attributed to a reduced uptake and utilization of the free fatty acids by the damaged liver. Recent studies by Spahn (7) appear to confirm this assumption of a utilization disorder of free fatty acids in the case of functional restriction of the liver. The poor tolerability of pure coffee could be explained thus, especially because caffein-fr coffee is tolerated significantly better.

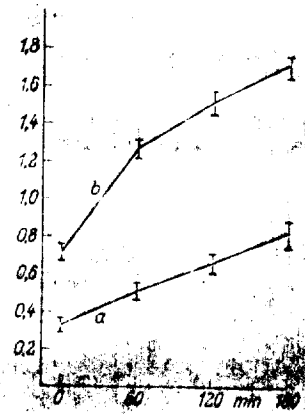
From these studies we can conclude that an increased fast level of free fatty acids points to a fat utilization disorder in the liver. In the case of patients with liver disease, a further separation of free fatty acids from the deposited fat can be expected, leading to a misproportion between available free fatty acids and the possibility of further processing in the liver. Thus, in all therapeutical considerations we should reckon in the fact that patients with liver disease should by no means be given caffein-containing beverages or medicines with their low-fat diets. In selecting an appropriate coffee, therefore, consideration should always be made of the absolute caffein content, and not any pretreatment, which has no effect on the separation of free fatty acids from the deposited fat.

Bibliography

1. *Alexander, R. H., S. J. Friedberg, M. D. Bogdonoff, E. H. Estes:* Metabolism 12 (1963), 197.
2. *Bellet, S., A. Kershbaum, J. Aspe:* Arch. int. Med. 116 (1965), 750.
3. *Dole, V. P., H. Meinertz:* J. biol. Chem. 235 (1960), 2595
4. *Little, J. A., H. M. Shanoff, A. Cesma, R. Yano:* Lancet I (1966), 732.
5. *Mortiaux, A., A. M. Dawson:* Gut 3 (1961), 304.
6. *Schön, H., W. Zeller:* Münch. med. Wschr. 104 (1962), 2433.
7. *Spahn, U.:* Klin. Wschr. 45 (1967), 530.
8. *Spitzer, J. J., I. M. Miller:* Proc. Soc. exp. Biol. (N.Y.) 92 (1956), 124.
9. *Wajchenberg, B. L., G. Hoxter, E. H. L. Mello, A. B. U. Castro, O. Nogueira.* Lancet I (1960), 1218.
10. *Zeller, W.:* Vortr. 17. Dtsch. Therapiewoche, Karlsruhe 1965.
11. [illegible] Vortr. 6. Gastroenterolog. Fortbildungskurs.
12. [illegible], *H. P. T. Ammon:* Z. Gastroenterol. 5 (1967), 84.







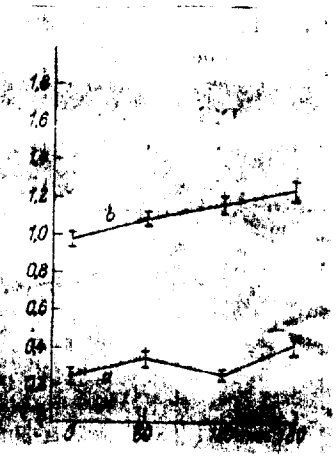


Figure 1 Figure 2 Figure 3 Figure 4

Figure 1. Experiments with Columbia coffee. a)healthy subject b) patient with liver disease

Figure 2. Experiments with steamed coffee. a) healthy subjects b) patients with liver disease

Figure 3. Experiments with coffee pretreated with CO_2. a) healthy subjects b) patients with liver disease

Figure 4. Experiments with caffein-free coffee. a) healthy subjects b) patients with liver disease

1. free fatty acids (µval/ml)

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Einfluß von Kaffee auf den Fettstoffwechsel

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Bei 44 leberkranken Patienten und 41 gesunden Versuchspersonen wurde die Wirkung verschieden vorbehandelter Bohnenkaffeesorten auf den Fettstoffwechsel untersucht und mit coffeinfreiem Kaffee verglichen. Die Mobilisierung der freien Fettsäuren aus dem Depotfett zeigte eine direkte Abhängigkeit vom absoluten Coffeingehalt der Kaffeemischung, unbehandelt oder behandelt. Der coffeinfreie Kaffee dagegen bewirkt keine Belastung des Fettstoffwechsels. Bei Leberkranken sollte die Zufuhr von Coffein bzw. coffeinhaltigen Getränken vermieden werden.

Neben den bislang bekannten Unverträglichkeitsreaktionen, wie Sodbrennen, Magenbeschwerden, Spasmen im Magen-Darm-Bereich, Unruhe, Herzklopfen und Schlafstörungen, führt der Kaffeegenuß auch zu einer direkten Beeinflussung des Fettstoffwechsels. *Bellet et al.* [2] hatten erstmals beobachtet, daß coffeinhaltiger Kaffee zu einem *Anstieg der freien Fettsäuren im Blut* führt. Nach neueren Untersuchungen soll der durch Coffeinwirkung bedingten Erhöhung der freien Fettsäuren im Plasma eine pathogenetische Bedeutung für verschiedene Erkrankungen zukommen. So wurde bei Patienten mit Koronarsklerose nach längerer Verabreichung von Kaffee eine signifikante Zunahme von Lipoproteiden und Gesamtcholesterin beobachtet, als deren Ursache die verstärkte

diskutiert wird [4]. Wir selbst konnten nachweisen, daß coffeinhaltiger Kaffee bei leberkranken Patienten gegenüber gesunden Versuchspersonen zu einem wesentlich stärkeren und länger anhaltenden Anstieg der freien Fettsäuren führt [12]. Diese Wirkung ist abhängig vom Coffeingehalt. Nach Verabreichung von coffeinfreiem Kaffee waren keine Veränderungen des Fettstoffwechsels erkennbar.

Für unsere weiteren Untersuchungen interessierte daher besonders die Frage, ob verschiedenartig vorbehandelte Kaffeemischungen die Wirkung von Bohnenkaffee auf den Fettstoffwechsel beeinflussen. Wir verglichen deshalb mehrere coffeinhaltige Kaffeesorten, wie Columbia-Kaffee, handelsüblichen nach *Lendrich* gedämpften Kaffee mit nach dem *Bach*-Verfahren mit Kohlensäure gewaschenem Kaffee und mit nach dem HAG-Verfahren entcoffeiniertem Kaffee.

Beobachtungsgut und Methodik

1. *Auswahl der Patienten*

Für die orale Applikation von coffeinhaltigem bzw. coffeinfreiem Kaffee wurden insgesamt 85 Versuchspersonen ausgewählt. Hierbei handelte es sich um 44 leberkranke Patienten und 41 gesunde Versuchspersonen, die in 8 Gruppen eingeteilt wurden.

2. *Versuche mit Columbia-Kaffee*

Die Gruppe I umfaßte 11 leberkranke Patienten und die Gruppe II 10 gesunde Versuchspersonen. Nach den analytischen Untersuchungen betrug die Coffeinmenge in 250 ml Aufguß von 20 g gemahlenem Kaffee 225 mg.

3. *Versuche mit gedämpftem Kaffee*

Die Gruppe III setzte sich aus 13 leberkranken Patienten und die Gruppe IV aus 10 gesunden Versuchspersonen zusammen. Die analytisch bestimmte Coffeinmenge betrug im Aufguß von 20 g Kaffee 193 mg.

Die Gruppe V bestand aus 10 leberkranken Patienten und Gruppe VI aus 9 gesunden Versuchspersonen. Im Aufguß von 20 g Kaffee waren 201 mg Coffein enthalten.

5. *Versuche mit coffeinfreiem Kaffee*

Die Gruppe VII umfaßte 12 leberkranke Patienten und die Gruppe VIII 10 gesunde Versuchspersonen. Der Coffeingehalt betrug in 20 g Kaffee 9 mg.

6. *Zubereitung des Kaffeeaufgusses*

20 g gemahlener Kaffee wurden mit 300 ml siedendem Wasser übergossen. Nach 7 Minuten wurde der Kaffee abgeseiht und von den Versuchspersonen ohne weitere Zutaten getrunken. Die getrunkene Flüssigkeitsmenge des Kaffeeaufgusses betrug 250 ml.

7. *Vorbereitung der Versuchspersonen*

Vor Versuchsbeginn wurden bei allen Versuchspersonen die Blutfettwerte (freie Fettsäuren, veresterte Fettsäuren und Gesamtcholesterin), Serumlabilitätstests (Blutkörperchensenkungsgeschwindigkeit, Takata, Thymol, Bilirubin und Elektrophorese), Leberfunktionsproben (Galaktose- und Bromthalein-Probe) und Serumfermentaktivität (SGPT, SGOT und LDH) bestimmt. Nach etwa 12stündigem Fasten wurde bei allen Versuchspersonen Blut mittels Venülen als Leerwert entnommen. Anschließend wurden 250 ml des entsprechenden Kaffeeaufgusses als Probetrunk gereicht und die Veränderungen des Blutfettspiegels über 360 Minuten verfolgt. Blutentnahmen erfolgten nach 30, 60, 120, 180, 240 und 360 Minuten. Bestimmt wurden Gesamtcholesterin und veresterte Fettsäuren nach einem früher mitgeteilten Verfahren [6] und die freien Fettsäuren nach *Dole et al.* [3].

Ergebnisse

Bei gesunden Versuchspersonen ist nach oraler Zufuhr von coffeinhaltigem Kaffee ein signifikanter Anstieg der

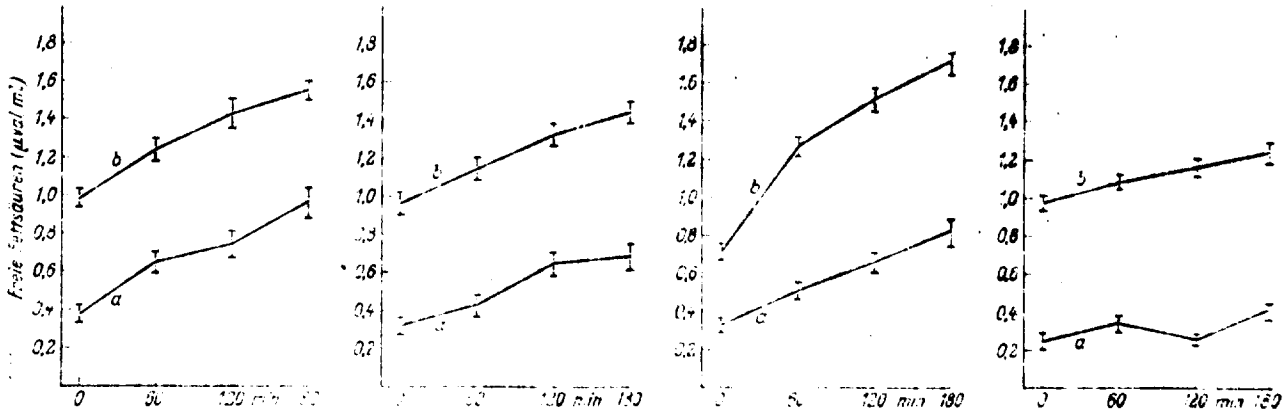

Abbildung 1 *Abbildung 2* *Abbildung 3* *Abbildung 4*

Abbildung 1. Versuche mit Columbia-Kaffee. a) Gesunde Versuchspersonen, b) leberkranke Patienten.

Abbildung 2. Versuche mit gedämpftem Kaffee. a) Gesunde Versuchspersonen, b) leberkranke Patienten.

Abbildung 3. Versuche mit CO_2-behandeltem Kaffee. a) Gesunde Versuchspersonen, b) leberkranke Patienten.

Abbildung 4. Versuche mit coffeinfreiem Kaffee. a) Gesunde Versuchspersonen, b) leberkranke Patienten.

freien Fettsäuren im Plasma zu beobachten. Entcoffeinierter Kaffee führt dagegen zu keiner signifikanten Änderung der freien Fettsäuren im Plasma.

Bei Verwendung von coffeinhaltigem *Columbia-Kaffee* nahm die Plasmakonzentration der freien Fettsäuren bei gesunden Versuchspersonen im Mittel von 0,41 auf 0,89 µval/ml nach 3 Stunden zu (*Abbildung 1*). Nach 5 Stunden fiel sie wieder auf den Ausgangswert ab. Bei den leberkranken Patienten lag der Durchschnittswert der freien Fettsäuren im Plasma mit 1,08 µval/ml erheblich über dem Kontrollwert der gesunden Versuchspersonen. Nach oraler Verabreichung von Columbia-Kaffee war ein Anstieg auf 1,65 µval/ml zu erkennen.

Gedämpfter Kaffee führte bei gesunden Versuchspersonen zu einem Anstieg der freien Fettsäuren von 0,32 auf 0,71 µval/ml (*Abbildung 2*) und bei leberkranken Patienten von 0,97 auf 1,39 µval/ml.

Nach mit *Kohlensäure vorbehandeltem Kaffee* stiegen die freien Fettsäuren im Plasma der gesunden Versuchspersonen von 0,37 auf 0,82 µval/ml (*Abbildung 3*) und bei leberkranken Patienten von 0,99 auf 1,58 µval/ml.

Coffeinfreier Kaffee führte zu keiner signifikanten Erhöhung der freien Fettsäuren bei gesunden Versuchspersonen (*Abbildung 4*) bzw. leberkranken Patienten.

Diskussion

Unsere Untersuchungen belegen erneut, daß bei leberkranken Patienten gegenüber gesunden Versuchspersonen ein erhöhter Nüchternspiegel an freien Fettsäuren im Plasma nachweisbar ist und nach oraler Zufuhr von coffeinhaltigem Kaffee ein wesentlich stärkerer und länger anhaltender Anstieg der freien Fettsäuren auftritt. Eine Gegenüberstellung von verschieden vorbehandelten Kaffeesorten und entcoffeiniertem Kaffee läßt deutlich erkennen, daß der Anstieg der freien Fettsäuren im Plasma direkt vom Coffeingehalt abhängig ist. Entsprechend seinem geringeren Coffeingehalt führt gedämpfter Kaffee zu einem geringeren Anstieg der freien Fettsäuren im Serum als z. B. Columbia- bzw. CO_2-behandelter Kaffee. Entcoffeinierter Kaffee läßt dagegen keine signifikanten Veränderungen des Serumspiegels der freien Fettsäuren erkennen.

Dem Stoffwechsel der freien Fettsäuren kommt insofern eine besondere Bedeutung zu, als durch diese Energieform im Organismus etwa 60—80 % des gesamten Kalorienbedarfs gedeckt werden. Wenngleich der höhere Ausgangswert der freien Fettsäuren im Plasma bei leberkranken Patienten keineswegs pathognomonisch für Lebererkrankungen ist, so sollte dennoch dieser Befund entsprechend berücksichtigt werden. Die Leber vermag normalerweise bis zu 50 % der aus dem Depotfett durch enzymatische Spaltung der Triglyceride mobilisierten freien Fettsäuren aufzunehmen. Der größte Teil der an Albumin gebundenen freien Fettsäuren wird wieder zu Triglyceriden und Phosphatiden verestert und in Form von β-Lipoproteiden an das Blut abgegeben oder über Acetyl-Coenzym A bis zu CO_2 und H_2O abgebaut. Diese enge Verknüpfung von Fettstoffwechsel und Leberfunktion läßt vermuten, daß bei eingeschränkter Leberfunktion auch die Aufnahme und Verwertung von freien Fettsäuren sowie der gesamte Fettstoffwechsel durch die geschädigte Leber bei leberkranken Patienten verringert ist. Diese Annahme wird durch frühere Untersuchungen gestützt, die eindeutig zeigen, daß bei leberkranken Patienten in durchschnittlich 60 % der Fälle erhöhte Blutfettwerte als Ausdruck einer begleitenden Fettstoffwechselstörung nachzuweisen sind [10, 11]. Andere Autoren sind zwischenzeitlich zu ähnlichen Ergebnissen gelangt [1, 7, 9].

Eine vermehrte Mobilisation von Depotfett [5] sowie ein gestörter intermediärer Kohlenhydratstoffwechsel bei leberkranken Patienten wurden zwischenzeitlich ebenfalls als Ursache des erhöhten Spiegels der freien Fettsäuren im Plasma diskutiert [8]. Am wahrscheinlichsten dürfte jedoch der erhöhte Nüchternspiegel der freien Fettsäuren bei Leberparenchymschädigung auf eine verringerte Aufnahme und Utilisation der freien Fettsäuren durch die geschädigte Leber zurückzuführen sein. Neuere Untersuchungen von *Spahn* [7] scheinen diese Annahme einer Verwertungsstörung von freien Fettsäuren bei Funktionseinschränkung der Leber zu bestätigen. Die schlechte Verträglichkeit von Bohnenkaffee könnte in dieser Richtung gedeutet werden, insbesondere auch deshalb, weil coffeinfreier Kaffee wesentlich besser vertragen wurde.

Aus diesen Untersuchungen kann gefolgert werden, daß ein erhöhter Nüchternspiegel an freien Fettsäuren auf eine Fettverwertungsstörung in der Leber hinweist. Bei leberkranken Patienten ist durch die Coffeinwirkung eine weitere Mobilisierung von freien Fettsäuren aus dem Depotfett zu erwarten, wodurch sich das Mißverhältnis zwischen Angebot von freien Fettsäuren und der Möglichkeit zur Weiterverarbeitung in der Leber noch weiter vergrößert. In allen therapeutischen Überlegungen sollte deshalb einkalkuliert werden, daß *leberkranken Patienten* neben einer fettarmen Diät *keinesfalls coffeinhaltige Getränke oder Medikamente gereicht werden dürfen*. Bei der Auswahl eines geeigneten Kaffeegetränks sollte daher stets der absolute Coffeingehalt und nicht irgendeine Vorbehandlung, die keinen Einfluß auf die Mobilisierung von freien Fettsäuren aus dem Depotfett ausübt, berücksichtigt werden.

Literatur

1. *Alexander, R. H., S. J. Friedberg, M. D. Bogdonoff, E. H. Estes:* Metabolism 12 (1963), 197.
2. *Beliet, S., A. Kershbaum, I. Asper:* Arch. int. Med. 116 (1965), 750.
3. *Dole, V. P., H. Meinertz:* J. biol. Chem. 235 (1960), 2595.
4. *Little, J. A., H. M. Shanoff, [illegible] Csima, R. Yano:* Lancet I (1966), 732.
5. *Mortimer, A., A. M. Dawson:* Gut 3 (1962), 351.
6. *Schon, H., W. Zeller:* Münch. med. Wschr. 101 (1962), 2133.
7. *Spahn, G.:* Klin. Wschr. 45 (1967), 530.
8. *Spitzer, J. J., I. M. Miller:* Proc. Soc. exp. Biol. (N.Y.) 92 (1956), 124.
9. *Wajchenberg, B. L., C. Hoxter, E. H. L. Mello, A. B. U. Cintra, O. Nogueira:* Lancet I (1960), 1218.
10. *Zeller, W.:* Vortr. 17. Dtsch. Therapiewoche, Karlsruhe 1965.
11. *Zeller, W.:* Vortr. 6. Gastroenterolog. Fortbildungskurs.
12. *Zeller, W., H. P. T. Ammon:* Z. Gastroenterol. 5 (1967), 84.

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Effects of Caffeine and Chlorpromazine on the Sexual Behavior of Male Rats

Although the effects of stimulant and tranquilizing drugs on sexual behavior are of considerable importance, the amount of systematic research on this problem has been negligible. Soulairac and Coppin-Monthillaud (*1*) have reported that after an injection of 30 mg of caffeine (and sodium benzoate) per kilogram, male albino rats decreased their latency to first sexual activity and increased their rate of copulation. The study described in this report (*2*) compares the effects of moderate doses of caffeine and chlorpromazine on the sexual performance of 17 150-day-old male hooded rats. The previous sexual behavior of the rats varied from complete impotence to extreme vigor.

two subjects which did not perform at all on any of the trials.)

Behavioral measures	No.	Caffeine	No drug	Chlorpromazine	Friedman analysis of variance
Latency to:					
(i) first mount	9	13.4	26.4	69.7	$\chi^2 = 10.1$*
(ii) first copulation	9	13.4	28.3	71.3	$\chi^2 = 9.5$*
(iii) ejaculation	6	436.7	589.2	528.3	$\chi^2 = 7.0$†
Frequency of:					
(i) mounts	15	4.1	4.7	3.9	Not significant
(ii) copulations	15	16.1	15.1	12.0	$\chi^2 = 7.4$†
Percentage of group that:					
(i) copulated	15	80	87	87	Not significant
(ii) ejaculated	15	47	50	47	Not significant
Copulatory rate:					
(i) for subjects that copulated	9	2.41	1.87	1.45	$\chi^2 = 8.7$*
(ii) for subjects that ejaculated	6	2.80	1.64	1.63	$\chi^2 = 9.2$*

* Significant beyond the 0.01 level of probability. † Significant beyond the 0.05 level of probability.

Each male was given four 15-minute tests, spaced at least 2 days apart. Males were injected with 20 mg of caffeine (and sodium benzoate) per kilogram before one test, with 1 mg of chlorpromazine per kilogram before another test, and with isotonic saline (no drug) before two tests (*3*). Drug tests were alternated with no-drug tests. All injections were given intraperitoneally, between 15 and 60 minutes before the start of the test; this time-interval was within the effective range of the drugs. The drugs were dissolved in the same volume of isotonic saline (1 ml/kg) that comprised the no-drug injections. The rats were randomly assigned to one of four groups, which differed in the sequence of drug and no-drug tests. During each of the four test sessions one group was under caffeine, one group was under chlorpromazine, and two groups were under no drug. By these procedures, the order of drug conditions was counterbalanced, and each drug condition was tested in each session.

The tests were conducted in a circular observation cage 30 in. in diameter. Each male was given 2 minutes alone in the cage before the introduction into the cage of a female which was in full behavioral heat. The sexual behavior of male rats consists of mounting the female and making a brief intromission (copulation). On some occasions the male may mount without accomplishing an intromission. After a number of intromissions, generally between 10 and 20, ejaculation occurs. The male withdraws from the female and shows no sexual interest for at least 15 seconds after each intromission and for at least several minutes after ejaculation.

Table 1 shows these measures of sexual activity, presented in terms of (i) latency in seconds of first occurrence after introduction of the female; (ii) frequency of occurrence during the 15-minute test; (iii) percentage of group performing the response; and (iv) copulatory rate. Copulatory rate is the average number of copulations per minute to ejaculation (or, if no ejaculation occurs, from the first copulation to the end of the test). These measures and the procedures are more fully described elsewhere (*4*). As is shown in Table 1, caffeine decreased the latencies to mount, copulate, and ejaculate and increased the frequency and rate of copulation. Chlorpromazine had opposite effects on all these measures except latency to ejaculation. Of interest is the failure of both drugs to alter significantly the percentage of subjects which copulated or ejaculated.

In order to test for the effects of the drugs on subsequent no-drug sessions, the tests following administration of caffeine were compared with the tests following administration of chlorpromazine. An additional 15-minute no-drug test was given to animals whose last regular test was with one of the drugs, in order to complete this procedure. The animals made more than twice as many mounts and 24 percent more copulations during the no-drug session following administration of chlorpromazine than during the no-drug session following administration of caffeine. The difference in number of mounts is statistically significant ($p < 0.05$ by Wilcoxon matched-pairs rank test). There were no reliable differences in the other measures of sexual behavior. Since the time interval between sessions was ample to allow the drug effects to dissipate, these results apparently point to differences in behavior learned under the two drugs. A more strenuous sexual response may have been learned under chlorpromazine to over-

come the depressant effects of the drug.

The wide range in sexual potency of our subjects was in part the result of antecedent conditions to which they were subjected in an experiment by one of us (P. G. Z.) (5). After weaning, 11 of the rats had been reared under unfavorable conditions (that is, they had been given electric shock for approaching a receptive female and had been subsequently reared in isolation, or they had been reared entirely in isolation). Six rats had been reared under more favorable conditions (that is, they had been given no electric shock when they were with a receptive female and had been reared in cohabitation with other males or with females). In order to increase the number of subjects in testing the differential effects of previous sexual experience, eight pretest rats were added to this comparative study. These animals had been reared under the same conditions as the others (five under favorable conditions, three under unfavorable ones) but were given four *ten*-minute tests and were not given a no-drug test between the two drug tests.

In the various measures of sexual performance, the depressant effects of chlorpromazine were found to be approximately equal among animals reared under these two sets of conditions. On the other hand, the stimulating effects of caffeine were evident only among animals reared under the more favorable conditions. These animals copulated almost twice as many times when they were under caffeine as when they were under no drug and showed an increment, under caffeine, in number of mounts, in copulatory rate, and in percentage that copulated and ejaculated. Among the animals reared under unfavorable conditions, caffeine produced only a slight increment in copulatory rate and a slight decrement in all of these other measures. The effect of rearing conditions on the caffeine-versus-no-drug difference was statistically significant for number of copulations ($p < 0.02$) and almost significant for number of mounts ($p < 0.10$), according to the Mann-Whitney rank test. The different effects of caffeine might be explained by the hypothesis that the stimulating effects of caffeine enhance whatever behavior tendency is dominant in the situation. If the conditions of rearing have been favorable, positive sexual responses will generally be dominant; if the conditions of rearing have been unfavorable, inhibitory or incompatible responses may be dominant.

The study described in the present report confirms and extends the finding by Soulairac and Coppin-Monthillaud of the stimulating effect of caffeine on sexual behavior—even of a smaller dose. A depressant effect of chlorpromazine was also found. The use of non-drug tests and of animals with variation in previous sexual experience permitted further observations on effects of the drugs.

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References and Notes

1. A. Soulairac and M. Coppin-Monthillaud, *J. Physiol. (Paris)* 43, 869 (1951).
2. This study was conducted while we were under predoctoral and postdoctoral fellowships, respectively, from the National Institutes of Health, U.S. Public Health Service. The costs for equipment and rats were financed in part by a National Science Foundation research grant.
3. Helpful advice on doses and injection procedures was given by N. J. Giarman of the department of pharmacology, Yale University.
4. F. A. Beach, A. C. Goldstein, G. A. Jacoby, Jr., *J. Comp. Physiol. Psychol.* 48, 173 (1955); also K. Larsson, *Conditioning and Sexual Behavior* (Almquist & Wiksell, Stockholm, 1956).
5. P. G. Zimbardo, unpublished.

23 September 1957

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What—Coffee, Too?

With the link between cigarettes and lung cancer already firmly established, it seemed only a matter of time before the epidemiologists would get around to showing that another breakfast tradition, the morning cup of coffee, may pose some dire hazards of its own. Coffee, in fact, has for some time been suspected as a promoter of heart disease; and now in the British journal Lancet, a Harvard researcher reports that it may also be linked to cancer of the bladder—particularly in women.

Dr. Philip Cole of the Harvard School of Public Health based his opinion on interviews with 445 patients with bladder cancer and a like number of healthy individuals in the Boston-Brockton area of Massachusetts. Cole's intent was to examine the relationship between bladder cancer, smoking and "occupational" hazards, but he also asked his subjects whether or not they drank coffee. The results showed that for women who drank one or more cups of coffee a day, the risk of developing bladder cancer was two and a half times greater than for those who drank less than this or none at all.

Suspect: Among men, Cole found the bladder-cancer risk was about 25 per cent higher in coffee drinkers, a figure that was not, in his words, "statistically significant." Yet, should a cause-and-effect relationship between coffee and bladder cancer be proved, the effect of coffee drinking on men would be just as great as it is on women, in terms of the total cancer problem. The reason: bladder cancer is four times more common in men than in women.

Cole doesn't know just what it is about coffee that could lead to bladder cancer. Caffeine is a likely suspect, he notes, because it has been shown to cause mutations in cells in tissue cultures. But he adds that the problem may not be related directly to coffee at all. It could be artificial sweeteners such as cyclamates which have, in fact, now been taken off the market because of evidence that they caused bladder cancer in animals. In view of this, Cole points out that no one should become alarmed because of his study until the association between coffee and cancer has been confirmed by other studies.

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DO HAVE SOME COFFEE BEFORE YOU LEAVE

When an Arabian abbot instructed his shepherd to pick the berries that had caused "nightlong friskiness and frolic" in his goats, he could not have foreseen the future that lay ahead for his discovery. It is estimated that today over a billion kilograms of coffee are consumed annually in the USA alone.

Modern pharmacological studies have confirmed the ancient belief that caffeine, the active ingredient of coffee, stimulates the skeletal muscles and the central nervous system. Although toxic reactions have been reported (*Cited in F.C.T.* 1968, 6, 389 & 674; *ibid* 1970, 8, 475) and are given further consideration in this review, no deaths have been recorded from the drinking of coffee and the practice continues to increase in popularity.

Recent studies offer some possible clues to the reasons for this popularity (*Nutrition Reviews* 1970, **28**, 38). Heavy users, drinking five or more cups of coffee daily, reported less wakefulness at night and less nervousness in the morning compared with light users who consumed only one or two cups daily (Goldstein & Kaizer, *Clin. Pharmac. Ther.* 1969, **10**, 477). Moreover, heavy consumers reported that they experienced irritability, an inability to work effectively, nervousness, lethargy and headache if they did not drink coffee in the morning. Similar conclusions were drawn from a double-blind study in which housewives were given decaffeinated coffee containing either added caffeine or a placebo (Goldstein *et al. ibid* 1969, **10**, 489).

Caffeine (1,3,7-trimethylxanthine) can be extracted from both coffee and tea and is also found in cola drinks. Because of the potential toxicity of the methylxanthines and the wide usage of products in which they are found, many workers have been engaged in projects to investigate the safety of prolonged and excessive drinking of coffee or tea.

Absorption and fate of ingested caffeine

The half-life of caffeine in man has been put at 3·5 hours (Axelrod & Reichenthal, *J. Pharmac. exp. Ther.* 1953, **107**, 519). By feeding [8-^{14}C]caffeine to rats and following the fate of the radioactive label, Czok *et al.* (*Z. Ernähr Wiss.* 1969, **9**, 103 & 109) demonstrated that both pure caffeine and caffeine ingested in coffee were absorbed quickly and their level in the serum soon reached a peak. The maximum urine concentration was found some 5 hours after administration. However, caffeine in a tea infusion was absorbed significantly more slowly, as shown by the longer time interval before peak serum and urine levels of radioactivity were reached. Excretion of caffeine was delayed in older rats, and also in young ones given a simultaneous dose of chlorogenic acid, although in this case absorption was relatively rapid. It thus appears that although tea contains less caffeine than coffee, its effects may be more pronounced, especially in the old, because of the relatively slow metabolism and elimination of its caffeine.

Mutagenicity

Several studies on the possible mutagenicity of caffeine have been carried out since an early report of its activity in Drosophila (*Cited in F.C.T.* 1968, **6**, 390). It has become clear that caffeine, a purine analogue, can interact with fully denatured DNA (Ts'o & Lu, *Proc. natn. Acad. Sci. U.S.A.* 1964, **51**, 17) and also with partially denatured DNA produced by ultraviolet irradiation (Domon *et al. Int. J. Radiat. Biol.* 1970, **17**, 395). The latter workers discovered that the binding of caffeine, unlike that of the natural bases, adenine and thymine, was greatest when only 50% of calf-thymus DNA was denatured. They concluded that the caffeine molecule binds to locally-denatured regions of the DNA and thus induces irregularities and abnormalities in the repair system which normally reconstitutes damaged DNA. Cleaver & Thomas (*Biochem. biophys. Res. Commun.* 1969, **36**, 203) have shown that caffeine can interfere with the *de novo* synthesis of DNA in ultraviolet-irradiated Chinese hamster cells. The exact mechanism of these effects cannot be fully elucidated until the controversy surrounding the DNA polymerases reaches a definite conclusion (*Nature, London* 1970, **226**, 685; *ibid* 1971, **229**, 155).

Currently, tests for caffeine mutagenicity are being carried out mainly in mice using the dominant lethal assay, which measures chromosomal damage and rearrangements in terms of zygote lethality and F_1-progeny sterility. In one such series of tests, administration of caffeine to male mice either as a single intraperitoneal dose of 168–240 mg/kg or in the

drinking water for 8 wk as a 0·05–0·4% solution failed to produce any mutagenic effect or to synergize the effects of known mutagens such as methyl methanesulphonate, TEPA or X-rays (Epstein *et al. Fd Cosmet. Toxicol.* 1970, **8**, 381). Similarly, Adler (*Humangenetik* 1969, **7**, 137) found that a single intraperitoneal dose of 250 mg caffeine/kg given to 29 male mice caused no significant increase in the incidence of post-implantation deaths among nearly 3500 embryos in over 370 females. However, copulation frequency was considerably decreased, especially in the first week after treatment. In cytogenetic studies in the same laboratory (Adler & Röhrborn, *ibid* 1969, **8**, 81), male mice given the caffeine equivalent of 2–400 cups of coffee daily for 37–50 weeks, showed no distinct evidence of chromosomal aberrations in the metaphase I figures of spermatogenesis.

Conflicting results are being obtained from work on Drosophila. This species is not a good one for mutagenicity testing because it is so far removed from man and is too susceptible to genetic change. In most of the caffeine studies, the incidence of chromosomal changes induced by the treatment was only two or three times higher than the spontaneous incidence and the statistical significance was based upon the very large sample numbers rather than on the magnitude of the effect. In general, it would appear that loss of sex chromosomes does occur both in adults and especially in larvae treated with caffeine (Bateman, *Mutation Res.* 1969, **7**, 475) but beyond this the data are too confused to form a basis for any constructive conclusions.

The possibility that caffeine may induce mutations in man is extremely unlikely. Studies of its effect on the rate of mitosis of human lymphocytes in tissue culture (Timson, *Br. J. Pharmac.* 1970, **38**, 731) have indicated that caffeine is indeed cytotoxic at high levels (10^{-2}M) and that it is a mitotic inhibitor at a concentration of 10^{-3} M. However, as the latter concentration would be reached in the total body water of a male adult only by the consumption of some 80 cups of coffee in a short time, the results of experiments at levels of 10^{-4} M are more important. At this level—equivalent to drinking eight cups of coffee in quick succession—caffeine was found to have a slight stimulatory action on the rate of mitosis of lymphocytes from female donors, while in those from males, mitosis was slightly inhibited or unaffected. But a small increase in mitotic index under conditions of tissue culture is of very questionable significance, and it is unlikely that coffee consumption even in more bizarre quantities would have a mutagenic effect.

Allergy to tea and coffee

Two recent reports have dealt with allergic responses to tea and coffee. Uragoda (*Br. J. ind. Med.* 1970, **27**, 181) has reported the case of a worker on a tea estate in Ceylon who developed asthma in the presence of tea fluff, a fine dust liberated during the manufacture of dried tea for packing, while Van Toorn (*Thorax* 1970, **25**, 399) has described a condition he calls 'coffee worker's lung'. This would appear to be related to 'farmer's lung' and 'pigeon breeder's lung' and is characterized by pulmonary lesions of a similar kind. Circulating antibodies against coffee-bean dust were detected in the serum of this patient who had worked in a coffee-roasting factory for over 20 years.

There has in the past been much controversy over the possible part played by chlorogenic acids in allergic reactions to coffee-bean dust (*Cited in F.C.T.* 1966, **4**, 243). These acids have been shown to survive the roasting process and remain in the beans, but recent allergenicity studies on roasted coffee extracts and on pure samples of the three chlorogenic acid isomers isolated from roasted coffee have not provided support for the theory that chlorogenic acids are involved in human allergy to coffee (Corse *et al. J. Sci. Fd Agric.* 1970, **21**, 164).

Metabolic and physiological effects

It is becoming increasingly apparent that, under certain conditions, caffeine induces the release of norepinephrine (noradrenaline) into the brain tissue of rats and guinea-pigs and also stimulates its synthesis (Berkowitz *et al. Eur. J. Pharmac.* 1970, **10**, 64). Furthermore, preliminary studies indicate that a similar release of epinephrine (adrenaline) from the adrenal medulla may also occur under the influence of caffeine. One of the steps in the release of catecholamines, such as epinephrine and norepinephrine, is the elevation or mobilization of intracellular calcium, and it has been suggested that such an increase may be one of the primary effects of caffeine.

Catecholamine release has been cited as a possible causative mechanism behind what was described as a case of coffee-induced headache (Harrie, *J. Am. med. Ass.* 1970, **213**, 628). Alternatively the persistent headaches reported by this patient may have been due to the niacin component of the coffee. Certainly, abstention from tea or coffee was sufficient to prevent further occurrence of the symptoms.

An increase in catecholamines also results in changes in glucose metabolism (Robison *et al. A. Rev. Biochem.* 1968, **37**, 149). The level of cyclic adenosine 3′,5′-monophosphate (AMP) increases the activity of the enzyme phosphokinase. This, in turn, stimulates the conversion of phosphorylase 'b' to the active form, phosphorylase 'a', which converts glycogen to glucose 1-phosphate. The resulting rise in glucose 1-phosphate level increases the rates of reaction in the Embden-Meyerhoff glycolytic pathway, the Krebs cycle and the hexose monophosphate (HMP) shunt and leads to a rise in oxygen consumption and a decrease in glucose tolerance.

Several of these effects have now been observed. A decrease in liver glycogen and a subsequent increase in blood glucose have been recorded in both normal and diabetic mice within some 2 hours of the subcutaneous injection of 25 mg caffeine/kg (Ammon & Estler, *Medna exp.* 1969, **19**, 161). The increase in blood glucose was much greater in the diabetic than in the normal mice and was not accounted for by a correspondingly greater fall in liver glycogen. This was apparently due to the fact that caffeine directly stimulates insulin secretion from the pancreas in non-diabetic animals.

Strubelt & Siegers (*Biochem. Pharmac.* 1969, **18**, 1207) have shown that an intraperitoneal injection of 60 mg caffeine/kg will increase the oxygen consumption of rats by 62%, and that only half of this consumption can be accounted for by enhanced mobility. The increase in oxygen consumption induced by 6·6 mg caffeine/kg was abolished by pretreatment with reserpine, while the calorigenic action of the larger dose was not affected by reserpine but was completely inhibited by propranolol. It seems that the action of small doses of caffeine is mediated exclusively by released catecholamines, while that of larger doses is also due partly to a direct calorigenic action of the drug. A decrease in the intravenous glucose tolerance of a group of pregnant non-diabetic women following the ingestion of two cups of coffee containing some 250–300 mg caffeine was observed by Goldman & Ovadia (*Obstet. Gynec., N.Y.* 1969, **33**, 214). However, the decrease was not great enough to constitute any danger to the mothers.

It is possible that the general stimulatory effect of caffeine may be mediated by the release of catecholamines, which may also be responsible for the reduction in lipogenesis reported earlier (*Cited in F.C.T.* 1968, **6**, 390).

However, the results of Dimitrov *et al.* (*J. Pharmac. exp. Ther.* 1969, **168**, 240) do not fit neatly into the general pattern outlined above. They found that caffeine sodium benzoate (CSB) in a dose of 2·5 mg/ml serum depressed the HMP shunt and glucose utilization as

measured by the *in vitro* disappearance of ^{14}C-labelled glucose in polymorphonuclear leucocytes. These effects were also apparent with a dose of 1·0 mg CSB/ml and the HMP shunt was affected by only 0·1 mg/ml. A similar effect on the HMP shunt was also demonstrated in leucocytes from a dog given an intravenous injection of 150 mg CSB/kg. It is possible that the apparent contradiction in these results could be due in part to the tissue selected or to the use of CSB rather than caffeine, although sodium benzoate itself failed under similar conditions to affect the activity of the HMP shunt.

The ability of caffeine to induce hepatic drug-metabolizing enzymes has already been reported (*Cited in F.C.T.* 1968, **6**, 674). Mitoma *et al. Archs Biochem. Biophys.* 1969, **134**, 434) have since shown that the stimulation of these enzymes by doses of 75 mg caffeine/kg given orally to rats twice daily for 3 days is inhibited by actinomycin D, an inhibitor of protein synthesis. This finding suggests that the caffeine acts by inducing synthesis of the microsomal enzymes. Unfortunately, however, further experiments on this caffeine-induced effect designed to detect the involvement of cyclic AMP, any increase in the incorporation of labelled amino acids into the microsomal proteins or changes in the permeability of the microsomal membrane were not particularly successful. Thus, it appears that caffeine may stimulate microsomal-enzyme activity without causing a net increase in enzyme synthesis. In a later study, Lombrozo & Mitoma (*Biochem. Pharmac.* 1970, **19**, 2317) found that although no increase in the cytochrome P_{450} content of the liver was detected, there was a change in the quality of the P_{450} haemoprotein in rats treated with caffeine and NADPH-cytochrome P_{450} reductase activity was slightly increased.

Caffeine and serum lipid levels

Increased concentrations of serum free fatty acids (FFA) following ingestion of caffeine were reported some years ago (*Cited in F.C.T.* 1966, **4**, 348). Zeller (*Medsche Klin.* 1968, **63**, 707) has also found, in tests on different types of coffee, that in both healthy adults and in patients suffering from liver disease the mobilization of FFA from depot fat is directly dependent on the caffeine content of the coffee. In this study, decaffeinated coffee had no effect on fat metabolism. Studies to determine the significance and consequences of these findings have investigated rises in serum cholesterol and correlations between the intake of caffeine and coronary disease.

Freeze-dried tea, decaffeinated coffee, coffee and pure caffeine have been fed to rats in amounts corresponding to a daily consumption of about 12 cups of the respective beverages by a 70-kg man (Naismith *et al. J. Nutr.* 1969, **97**, 375). Plasma triglyceride, phospholipid and cholesterol levels were comparable in the control and decaffeinated-coffee groups after 54 days and the changes were insignificant in the group receiving dried tea. However, the animals given freeze-dried coffee or caffeine had significantly higher levels of plasma cholesterol and phospholipid than the remaining groups and the concentration of plasma triglycerides was depressed. The magnitude of the changes in serum lipid levels was thus roughly proportional to the amount of caffeine in the supplemented diets. In a second experiment, replacement of the starch in the basal diet by sucrose caused a significant rise in the levels of plasma triglycerides. An independent comment on this paper (*Nutrition Reviews* 1969, **28**, 204) points out that there was a wide discrepancy in the plasma cholesterol levels of control groups in the first and second experiments, although the conditions were very similar. This point was not discussed by the authors but makes it difficult to assess the real relevance of the paper.

This same group (Naismith *et al. Nutr. Metabol.* 1970, **12**, 144) has also reported a related

study carried out in healthy male and female volunteers who normally consumed on average about 560 mg caffeine/day in tea and coffee. After 14 days on decaffeinated coffee (with a total caffeine intake of only 12 mg/day), there was a fall in blood glucose, a small rise in plasma cholesterol and phospholipid and a marked drop in plasma triglycerides. Consumption of 875 mg caffeine/day in coffee for a subsequent 20-day period had no effect on blood glucose, but reversed the changes in plasma lipids. The human response to caffeine demonstrated in this study was thus very different from that found in the rat.

Ammon & Estler (*loc. cit.*) have reported that subcutaneous injection of 50 mg caffeine/kg to mice resulted in rises in serum FFA and total liver fatty acids some 2 hours after treatment. These effects were considerably exaggerated in diabetic mice, in which the equilibrium between lipolysis and lipogenesis is disturbed further by caffeine treatment, so that large quantities of ketone bodies are formed from the excess FA in the liver. The increase in hepatic glycogen breakdown in diabetic mice is probably due, at least in part, to the raising of the cyclic AMP content by caffeine.

A critical review of this aspect of caffeine toxicity and some comments on the contradictory nature of some of the findings were published by Heyden (*Z. Ernähr Wiss.* 1969, **9**, 388), who argued that increases in FFA following catecholamine release commonly result from extreme physical exercise, cigarette smoking and emotional stress, although they are not generally associated with a subsequent rise in cholesterol levels. Little *et al.* (*Lancet* 1966, **i**, 732) showed that although a group of patients with coronary disease had raised serum lipid levels, there was no evidence to suggest that cholesterol levels had risen or that these patients drank more tea and coffee than those in a control group. On the other hand, Paul *et al.* (*Circulation* 1963, **28**, 20) demonstrated a significant correlation between coffee consumption and the later development of coronary disease. Again, however, serum cholesterol levels were normal. Heyden (*loc. cit.*) concludes from a study of the literature and from his own work on rabbits that temporary increases in FFA after coffee ingestion are not necessarily followed by rises in serum cholesterol or by an increase in the incidence of atherosclerosis. This conclusion is in direct contrast to that of Young *et al.* (*Nature, Lond.* 1967, **216**, 1015) who, in demonstrating that in rabbits tea ingestion counteracted to some extent the effect of a high fat diet on serum lipoproteins, also showed that the degree of atherosclerosis was proportional to the lipoprotein concentration.

Further evidence that methylxanthines may influence the heart has come from Strubelt *et al.* (*Arzneimitel-Forsch.* 1970, **20**, 473). Severe necrosis of the cardiac muscle was apparent in all treated animals 16 hours after administration of 100 mg theophylline/kg to a group of rats, but a similar dose of caffeine resulted in a less severe necrosis of the myocardium in only 20% of the treated rats. Although FFA levels rose, there was no change in serum cholesterol concentration. A slightly different approach by Ammon & Estler (*ibid* 1970, **20**, 471) demonstrated that 40 mg caffeine/kg given intravenously to anaesthetized rats increased the heart rate and blood pressure, especially in older animals.

It is clear that the action of caffeine on the heart is by no means insignificant and research must be continued until a definite conclusion as to its effect can be made. Muller & Vernikos-Danellis (*J. Pharmac. exp. Ther.* 1970, **171**, 153) have shown that the toxicity of caffeine, as measured by its LD_{50} in mice, is significantly increased by stress (resulting from dehydration or temperature changes of about 7°C) and it can also be affected by total food intake (*Cited in F.C.T.* 1967, **5**, 424). Another question has been raised by a recent finding of an apparent association between coffee drinking and cancer of the lower urinary tract (Cole, *Lancet* 1971, **i**, 1335). This apparent connexion came to light during an investigation into the

possible roles of cigarette smoking and occupational exposures in the aetiology of bladder cancer; the relative risk of the disease was found to be 1·24 among male and 2·58 among female coffee drinkers, compared with 1·0 among non-coffee-drinkers of either sex. The author stresses that no conclusion can be drawn from this finding beyond the fact that the relationship warrants further investigation. Timson (*Lancet* 1971, **ii**, 212) considering this finding in relation to the sex difference in the effect of caffeine on the mitotic rate in human lymphocytes (see p. 78 of this article), has suggested that while coffee may contain some carcinogens active in the bladder, the caffeine content could possibly have a secondary effect on bladder-cancer incidence through its action on the rate of mitosis of the malignant cells. This is again only speculation, but it provides food for thought and it is clear that in connexion with this as well as the other aspects mentioned above, there is still scope for further work on caffeine.

Heppel-'47
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The Lipotropic Activity of Caffeine, Theobromine and Theophylline

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Introduction

Evidence has been available for some time that the methylated purines are at least partially demethylated in the animal body. The older German literature is summarized in Cushny's textbook (1). Myers and Hanzal (2) first showed that caffeine and theophylline are oxidized and partially demethylated in the animal. Bernheim and Bernheim (3) studied the process *in vitro*. They found that liver slices of rabbit, guinea pig and rat were able to oxidize caffeine and theophylline. Theophylline was probably partially demethylated but experimental limitations made it impossible to decide whether caffeine was demethylated by their preparations.

If caffeine, theobromine and theophylline can be demethylated, the question arises whether this process can effectively contribute to the animal's requirements for labile methyl groups. Moyer and du Vigneaud (4) found that caffeine did not permit growth of rats on a methionine- and choline-free diet containing homocystine. Mills and Cottingham (5) observed that caffeine protected against hemorrhagic nephritis in rats due to choline deficiency but it was somewhat less effective than methionine. They also followed the growth of rats in simulated tropical environments. Under these conditions the requirements of choline for growth were increased. Methionine seemed well able to replace choline in supporting growth, but with caffeine in place of choline growth was retarded.

In the present study male weanling rats were fed a low protein-high fat diet deficient in choline. Some of the animals received dietary sup-

HORST, K., AND W. L. JENKINS 1935 The effect of caffeine, coffee and decaffeinated coffee upon blood pressure, pulse rate and simple reaction time of men of various ages. J. Pharm. and Exp. Ther., *53:* 385.

SCHULTE, J. W., E. C. REIF, J. A. BACHER, JR., W. S. LAWRENCE AND M. L. TAINTER 1941 Further study of central stimulation from sympathomimetic amines. J. Pharm. and Exp. Ther., *71:* 62.

SCHULTE, J. W., M. L. TAINTER AND J. M. DILLE 1939 Comparison of different types of central stimulation from analeptics. Proc. Soc. Exp. Biol. Med., *42:* 242.

the mechanisms involved. The only contribution we can offer at the present time with regard to the question is of a negative nature as stated in the summary.

SUMMARY

1. The modification of the action of caffeine by sucrose is not specific to this food material but is shared by foods of an entirely different nature, namely, protein and fat.

2. It is not due to an interference with absorption for, as shown in experiments 3 and 4, the effect was observed when caffeine was injected intraperitoneally and also when sugar was given after caffeine had been absorbed and had produced its characteristic action.

3. It cannot be accounted for by the mere presence of other material regardless of its nature in the gastro-intestinal tract, for agar administered simultaneously with caffeine had no neutralizing action.

In respect to the lethal dose of caffeine our results differ from those of Schulte and his associates ('39). A dose of 40 mg caffeine/kg proved fatal in 1 of their 10 animals. Since 10 mg/kg sufficed to increase spontaneous activity it was concluded that the margin of safety between the barely effective and the convulsive or fatal dose was 4 times. In the experiments reported in this paper and in numerous other experiments we have administered 75 mg caffeine/kg over 9000 times to 145 different rats without a single fatality.

LITERATURE CITED

CHAUCARD, P. 1945 Inhibition par les sucres de l'effét excitant qu'exercent les bas puriques sur le système nerveux. Compt. rend. de soc. de Biol., *139:* 12.

CHAUCARD, P., H. MAZOUE AND R. LECOQ 1945 Correction par les sucres des effets excitant de la caféine ou de la théobromine sur le système nerveux. Bull. Acad. de Med. Paris, *129:* 456.

CHENEY, RALPH H. 1935 Comparative effect of caffeine per se and a caffeine beverage (coffee) upon the reaction time in normal young adults. J. Pharm. and Exp. Ther., *53:* 304.

HOLLINGWORTH, H. L. 1912 The influence of caffeine on mental and motor efficiency. Arch. Psychology, no. 22, 1.

the activity of the animals when given 20 mg caffeine/kg in aqueous solution by stomach tube and when given the same amount of caffeine dissolved in 10% agar. Each value plotted is an average of 30 observations.

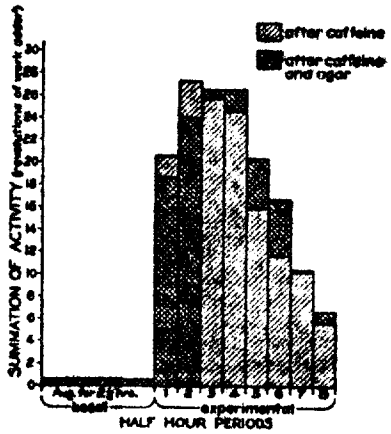

Fig. 7 Spontaneous activity following the administration of caffeine in water and caffeine in agar.

DISCUSSION

The original suggestion for the pursuit of this investigation arose in the course of another study still in progress which seemed to indicate that certain physiological actions of caffeine might be modified by the simultaneous ingestion of sucrose. The present study is concerned with a different underlying physiological mechanism.

While these experiments were under way it came to our attention that it had been reported by Chaucard ('45) and in another paper by the same author and his associates (Chaucard, Mazoue and Lecoq, '45) that the chronaxie of nerve fibers is affected by caffeine and that this effect is specifically neutralized by the oral administration of the sugars: lactose, sucrose, galactose and glucose.

When it had been proved to our satisfaction that the action of caffeine on the central nervous system can be modified by the administration of sucrose, our interest was aroused in

caffeine in oil are presented in figure 6. Each value from which the nomogram was constructed is an average of 60 experiments.

From this experiment and the preceding one it is apparent that the modifying effect on the action of caffeine which was observed originally in our studies with sucrose is not specific for sucrose but is shared by the other major foodstuffs, namely, protein and fat.

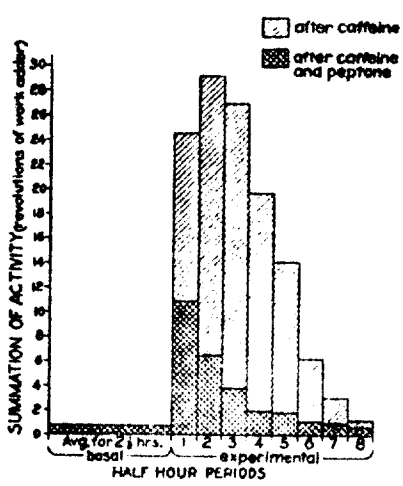

Figure 5

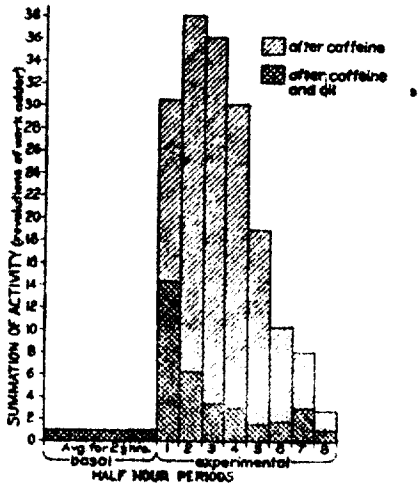

Figure 6

Fig. 5 Reduction in the stimulating action of caffeine by the simultaneous administration of peptone.

Fig. 6 The effect of the simultaneous administration of vegetable oil on the stimulating action of caffeine.

Experiment 7: Showing that the increase in spontaneous activity caused by caffeine is not affected by the simultaneous administration of inert material

There remained to be considered the possibility that the mere presence of materials other than caffeine in the gastrointestinal tract, regardless of their nature, might have an effect similar to that produced by the foodstuffs. This experiment, the results of which are presented in figure 7, shows that this is not the case. There was no significant difference in

During the first half hour after intraperitoneal injection of caffeine the activity was practically the same as in the preceding experiments in which caffeine was administered by stomach tube. In the second half hour there was a further increase in activity which continued at this high level during the following half hour period. From this time on there was a gradual return toward the basal level.

The oral administration of sugar neutralized to a large extent the stimulating action of caffeine that had been injected intraperitoneally.

Experiment 5: Showing that the increase in spontaneous activity induced by caffeine is reduced by the simultaneous administration of peptone

Further speculation on the mechanism whereby sucrose modifies the action of caffeine suggested the advisability of determining whether this effect was specific with respect to sucrose or common to other food materials of an entirely different nature. This experiment and experiment 6 were designed for this purpose.

Twelve animals that had not been used in the previous experiments were selected from the colony. The procedure was the same as in experiment 2 except that an aqueous solution containing 10 gm peptone/kg body weight was given instead of the sucrose solution.

The results presented in figure 5 show that peptone had an effect similar to that of sucrose in reducing the increase in activity caused by caffeine.

Experiment 6: Showing that administration of vegetable oil simultaneously with caffeine reduces the increased activity resulting from caffeine

Results similar to those in experiment 5 were obtained in the present experiment in which 20 mg caffeine dissolved in 3.75 ml vegetable oil was administered by stomach tube to 12 animals. The comparative effects of caffeine in water and

lowed in the next half hour period by a definitely greater decrease in activity than when water alone was given.

Experiment 4: Showing that caffeine injected intraperitoneally increases spontaneous activity which in turn is reduced by the oral administration of sucrose

This experiment is offered as confirmatory evidence that the effect of sucrose on the action of caffeine is not due to an interference with absorption. The observations were made on the same animals as in the preceding experiment.

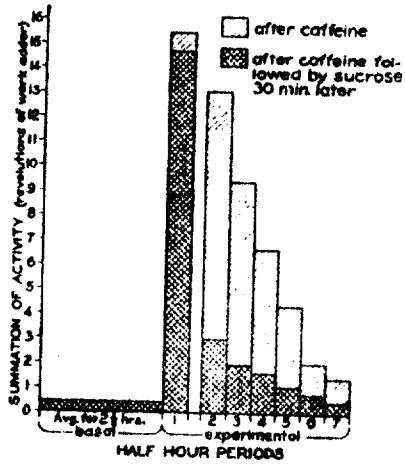

Figure 3

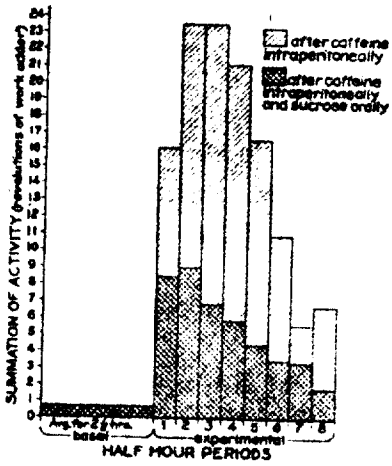

Figure 4

Fig. 3 Reduction in the increased activity produced by caffeine when sucrose was administered 30 minutes after caffeine.

Fig. 4 The action of the oral administration of sugar on the increase in spontaneous activity caused by the intraperitoneal injection of caffeine.

An aqueous solution containing 20 mg caffeine/kg was injected intraperitoneally into all the animals at the conclusion of the basal period. Immediately after the injection 1 group was given sucrose by stomach tube and the other water only. On alternate days this procedure was reversed.

The average values of the data obtained are plotted in figure 4. Each average was derived from 36 experiments.

A comparison of the activity when caffeine alone was given with that which occurred after the simultaneous administration of caffeine and sucrose (fig. 2) shows that the sugar neutralized to a large extent the action of caffeine.

In other experiments, the results of which need not be given in detail, spontaneous activity after the administration of a sucrose solution alone was found to be practically the same as when plain water was given.

Experiment 3: Showing that the increase in activity caused by caffeine can be reduced by administering sucrose 30 minutes after giving caffeine

When it had been definitely shown in the preceding experiments that the ingestion of sucrose had a modifying effect on the action of caffeine, speculation arose as to the mechanism involved. To determine whether the effect of the simultaneous administration of sugar might be due to an interference with the absorption of caffeine the following experiments were undertaken.

The animals used in this experiment were the same as in experiment 2. The procedure and the amount of caffeine and sucrose administered were also the same with the exception that all the animals were given caffeine immediately after the basal period. Thirty minutes later those under test conditions were given the sucrose solution by stomach tube and those under control conditions were given the same amount of water. There were 48 observations under each set of conditions.

The area of cross-hatched lines in figure 3 for the first half hour after the basal period represents the activity of the animals on the days when they were given sucrose after this first half hour period; the hatched lines, the activity on the days when they were given water (control experiments). Within 30 minutes the caffeine had been absorbed and was exerting its maximum effect, as indicated by the activity of the animals at this time. Administration of sucrose was fol-