LARD AND LARD OIL #72

Monograph on Lard & Lard Oil

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M O N O G R A P H

O N

L A R D A N D L A R D O I L

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LARD AND LARD OIL

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LARD

Summary

In both its refined and natural states, lard has been a part of the human diet for thousands of years. It has been the subject of considerable research in the past and its inclusion on the GRAS list has led to the current report.

The digestion and metabolism of lard has been fairly well elucidated. In studies with chickens, digestibility coefficients ranging from 84 to 95% have been determined for lard (293, 488, 312). The metabolizable energy value of lard for chicks was reported by Peterson and Vik-Mo (293) to be 7.92 kcal/g and by Renner and Hill (488) to be 3980 Cal/lb. Hydrolysis of lard to the form of free fatty acids reduced the digestibility to 65% (488). Carlson and Bayley (54) determined the digestibility coefficient of lard to be 81% when fed to young pigs as 10% of the diet. In tests with human volunteers consuming 50-115 g of lard daily, Langworthy (466) determined the digestibility of lard to be 97%.

According to Kayden et al. (462) analysis of intestinal lumen contents of man after fat feeding has shown a separation of glycerides into two phases: an oil phase which contains mainly diglycerides and triglycerides and some fatty acid, and a micellar phase which is a combination of bile salts, free fatty acids, and monoglycerides. From the micellar solutions monoglycerides and free fatty acids are absorbed through the intestinal wall, leaving the bile salts within the lumen to form additional micelles. The fate of the free fatty acids within the mucosal cell is determined by their chain length and saturation; those of less than 12 carbons are preferentially absorbed by rats directly into the portal blood, whereas longer chain fatty acids are incorporated mainly into triglycerides, and appear in lymph chylomicrons. Monoglycerides within epithelial cells may be esterified to di- and triglycerides or cleaved to glycerol and free fatty acids. The 2-monoglycerides are esterified especially rapidly to triglycerides, but esterification of 1-monoglycerides lags and these are more likely to undergo hydrolysis particularly if the fatty acids are of short chain length.

Fatty acids play an important role as an energy source. Their oxidation is prominent in higher animals which can store large amounts of neutral fat as a fuel reserve. It is estimated that fatty acid oxidation provides at least half of the oxidative energy in the liver, kidneys, heart muscle and resting skeletal muscle. Fatty acids undergoing oxidation in tissues of higher animals come either from extracellular fluid or from endogenous intracellular lipids. Vertebrate blood contains considerable amounts of triacylglycols but very small amounts of free fatty acids. The major endogenous source of fatty acids for fuel is storage fat, in the form of fat droplets in the cytoplasm, which consists largely of triacylglycerol. Before the fatty acids can undergo activation and oxidation, the triacylglycerols first must undergo hydrolysis

by intracellular lipases to yield free fatty acids and glycerols. Free fatty acids are therefore derived either from the blood stream, in which they are transported bound to serum albumin, or prior hydrolyses of intracellular lipids. They are first activated by esterification with CoA to form acyl CoA esters of the outer mitochondrial membrane. Then the fatty acyl group from CoA is carried across the inner membrane by the carrier molecule carnitine. Finally the fatty acyl group is transferred from carnitine to intramitochondrial CoA: through several enzymatic reactions. A two-carbon fragment of the fatty acid is removed as acetyl CoA and two pairs of hydrogen atoms are given to specific acceptors. A long chain fatty acid will go through several spirals. Thus the 16-carbon palmitic acid undergoes a total of 7 such cycles, to yield 8 molecules of acetyl CoA and 14 pairs of hydrogen atoms. The acetyl CoA formed as a product of the fatty acid oxidation will then enter the tricarboxylic acid cycle, which is the final common pathway of oxidation catabolism of all fuel molecules in aerobic cells (468).

Houssay and Martinez (160) reported that the toxic and diabetogenic action of alloxan increases in rats fed a high-lard (34%) diet. Kline et al. (195) found that feeding a high-lard (20%) diet to rats increased the carcinogenicity of p-dimethylaminoazobenzene. Leiter and Shear (212) observed that when used as the vehicle for single s.c. injections of 3,4-benzpyrene, lard residue excercised a striking retardation of tumor genesis by benzpyrene, while lard filtrate promoted rapid tumor production in a high proportion of the test animals.

Two teams of researchers, Velasco and Pancaldi (508) and Vinson and Cerecedo (392), have studied the influence of high-lard diets on the reproduction of rats.

Velasco and Pancaldi (508) reported that 1 male and 4 female rats maintained on bread, milk and water ad libitum and given a supplement of 4-5 g of lard daily exhibited rather stunted growth and periodically recurring disturbances of their physiology: a few days after initiation of the diet containing fat the rats began to lose their appetite, to become emaciated, their snouts became somewhat swollen, the eyelids rather edematous and reddened with thinner eyelashes, the fur rumpled and the external genital organs extensively reddened and swollen. These rats gave birth to babies in a poor state of general health and nutrition, weakened with a parched skin, and displaying a more or less pronounced state of adiposis in the liver.

In a follow-up study by Velasco and Pancaldi (508), 3 female rats were maintained on the high-lard diet throughout the period of pregnancy and lactation. Observations on these rats and the litters they produced confirmed the findings of the previous study. The workers further observed that, in contrast to the control litters, the young of the treated rats displayed stunted growth, with parched and wrinkled skin, a cyanotic color, and sluggish movements. These babies began to grow hair and opened their eyes several days later than the controls. At autopsy (25-27 days of age) the babies from the rats fed high-lard diets were considerably smaller than the controls and exhibited an accentuated state of adiposis in the liver.

In the experiments performed by Vinson and Cerecedo (392), rats raised for four generations on purified diets containing 2, 5, or 10% lard exhibited normal growth, but mothers on these diets invariably lost weight during lactation and weaned fewer young than the controls. Supplementary lard supplied to the rats ad libitum was eaten ravenously (as much as 8 g/day) with no improvement in lactation. In fact, the mothers fed the lard supplement destroyed their young during lactation.

Silberberg and Silberberg conducted a series of experiments on the effect of a high-lard (25%) diet on the development of arthritis in mice. They found that in mice of strain C57BL, prolonged feeding of the high-lard diet accelerated articular aging and accentuated the evolution of osteoarthritis (496, 344, 343). The intensity of the effect was found to depend upon strain (499), sex (497), and age (496, 499). In similar studies by Sokoloff and Mickelsen (502), a 25% lard diet was found to have no deleterious effects on the development of degenerative joint disease in male DBA/2JN mice.

Herting and Crain (449) reported that the maintenance of Holtzman rats for 16 weeks on a diet containing 50% fully hydrogenated lard resulted in the production of an unusual foreign-body type reaction. Rats given 50% untreated lard, however, exhibited no such reaction.

Several studies on the carcinogenicity of lard have been reported. Barry and Cook (22) observed the production of 2 non-malignant, injection-site tumors in 2 of 10 rats injected with lard; of 20 mice injected none developed tumors. In a study by Burrows et al. (49), 7 of 134 rats developed injection-site tumors, which were described by the authors as a low grade of malignant neoplasia. In further testing by Burrows et al. (49), 20 mice and 20 rats repeatedly subjected to i.p. injections of lard or lard plus 5% oleic acid developed no injection-site tumors. Andervont (423) reported that of 190 mice given a total of 1.0 ml of lard in three s.c. injections over a period of a month, none developed tumors during the 27 week observation period.

No acute toxicity data is available on lard. One short-term and three long-term studies have been reported. In a 6-week feeding study in which lard was fed to rats at dietary levels ranging from 10 to 73 cal%, Thomasson (367) found that the food-efficiency of lard is comparable to that of summer butterfat and that feeding lard at these levels produces no adverse effects on mortality. In a long-term study utilizing 463 mice, Silberberg and Silberberg (498) found that a 24% lard diet significantly increased the mortality and shortened the life span of male, but not female, mice of the C57 strain; male DBA mice were similarly affected but to a lesser degree. A follow-up study by Silberberg and Silberberg (500) showed that the effect of feeding a high-fat (25% lard) diet for 5 months at a time depended upon the period of life during which the fat-enriched diet was consumed. Given during the period of growth, the high-fat ration did not alter the life span; in young adults it had a beneficial effect, while given to old animals it exerted a slightly injurious action on the life span (500). The only effect of lard observed by Siedler et al. (342) in a 57-week feeding study with rats was the tendency toward increased serum and liver cholesterol.

The breakdown of lard is aptly introduced by the following remarks by Poling et al. (298):

> "Edible fats undergo chemical and physical changes when exposed to heat/or oxygen or both and, if the treatment is severe enough, some of the products may be harmful to experimental animals when fed under appropriate conditions. The nature and extent of the changes have been investigated extensively during the last few years, yet because of the complexity of the mixtures, there is still much to be learned regarding the composition and the biological effects of heated or oxidized fats. Three general types of chemical changes occur: oxidation, cleavage to smaller compounds and polymerization.
>
> There is considerable evidence that the treatments to which edible fats may be subjected during practical home or institutional use do not cause the formation of deleterious substances in amounts sufficient to impair health. On the other hand, several teams of scientists have observed adverse biological effects when fats which had been severaly heated or extensively oxidized were fed or injected."

The reports by Poling et al. (298) and Alfin-Slater (5) included in this monograph relate studies in which heated lard was fed to rats for long periods without the production of adverse effects.

Other reports, however, are included which describe manifestations of toxicity resultant from feeding heat-treated lard to rats. Upon feeding heated lard, Beck and Peacock (428) observed the development of signs of avitaminosis-A and some cases of ulceration and papillomatosis of the fore-stomach. Peacock and Beck (286) reported that 4 out of 71 rats surviving more than 300 days on a heated-lard diet developed multiple mesenteric sarcomata. Kennelly and Quackenbush (188) found that heated lard interfered with reproduction when fed in the diet, but not when fed by dropper separately from the diet, indicating that the oxidized fat exerted no toxic effects itself, but destroyed some essential component of the non-lipid portion of the diet. Morris (247) reported that the consumption of heated lard severely inhibited the growth of rats and resulted in the development of a progressive type of paralysis having characteristics of vitamin E deficiency. Upon autopsy, Morris (247) observed chronic gastric ulcers in a few of the rats and cirrhosis of the liver in many.

LARD

Chemical Information

I. Nomenclature

A. Common Names

1. Lard
2. Adeps
3. Axungia porci

B. Chemical Names

None

C. Trade Names

None

D. Chemical Abstracts Registry Number

977001-57-2

II. Empirical Formula

Not Applicable

III. Structural Formula

Not Applicable

IV. Molecular Weight

Not Applicable

V. Specifications

A. Chemical

Lips and Grant determined the characteristics of 33 samples of lard collected from 26 packing plants in Canada. The values obtained are presented in Table 1. In Table 2 some of these values are compared with other reported values (219).

TABLE 1

MEAN VALUES AND RANGES FOR MEASURED PROPERTIES OF CANADIAN LARD

Measurement	Mean	Range	Measurement	Mean	Range
Iodine number	58.7	53.1–65.3	Swift stability, hr. at 97.8 °C	3.5	0.3–9.0
Saponification number	193.9	184.2–202.2	Ferrometric peroxide, m.e. per kgm.	9.7	0.0–73.2
Melting point, °C.	43.5	39.3–47.9	Iodimetric peroxide, ml. of 0.002 N thiosulphate per gm.	1.6	0.0–14.2
Smoke point, °F.	382	345–438	Kreis test, extinction coefficients	9.9	3.7–20.7
Yellow colour, Lovibond units	8.8	3.0–22.3	Stamm test, extinction coefficients	2.3	0.0–19.9
Red colour, Lovibond units	1.6	0.4–3.1	Alpha-dicarbonyl test, extinction coefficients	3.4	0.8–9.9
Blue colour, Lovibond units	0.2	0.0–1.2	Free fatty acid, as % oleic	0.4	0.1–0.9
Saturated acids*, %	45.6	—	Fluorescence, photofluorometer units	79.2	39.8–146.0
Oleic acid*, %	44.7	38.6–51.8			
Linoleic acid*, %	8.7	4.6–11.3			
Linolenic acid*, %	0.6	0.4–1.1			
Arachidonic acid*, %	0.4	0.3–0.5			
Unsaponifiable matter, %	0.43	0.20–1.70			
Storage life, weeks at 26.7° C.	9.2	2.0–22.0			

Expressed as % of total acids.

TABLE 2

A COMPARISON OF VALUES FOR MEASURED PROPERTIES OF CANADIAN LARD WITH OTHER REPORTED AND RECOMMENDED VALUES

Measurement	Comparative values			
	Canadian		American	Other
	Mean	Range		
Iodine number	58.7	53.1–65.3	27 samples, 64.6 (4) Recommended, 46–70 (3)	General, 46–66 (22, p. 136) English, 108 samples, 57–73 (56) Portuguese, 30 samples, 63.9 (46)
Saponification number	193.9	184.2–202.2	Recommended, 195–202 (3)	General, 193–200 (22, p. 136) Portuguese, 30 samples, 195.8 (46)
Melting point, °C.	43.5	39.3–47.9	—	General, 28–48 (22, p. 136)
Smoke point, °F.	382	345–438	6 samples, 371 (59)	—
Saturated acids*, %	45.6	—	27 samples, 36.8 (4)	Portuguese, 30 samples, 38.5 (46)
Oleic acid*, %	44.7	38.6–51.8	27 samples, 51.5 (4)	Portuguese, 30 samples, 49.2 (46)
Linoleic acid*, %	8.7	4.6–11.3	27 samples, 11.7 (4)	Portuguese, 30 samples, 12.5 (46)
Arachidonic acid*, %	0.4	0.3–0.5	0.02–0.21 (15) 0.31–0.40 (11) 0.2 –0.6 (6)	
Unsaponifiable matter, %	0.43	0.20–1.70	Recommended, 1.0 max. (3)	General, 0.2–0.4 (22, p. 136; 29, p. 135) Portuguese, 30 samples, 0.14 (46)
Swift stability, hr. at 97.8 °C.	3.5	0.3–9.0	7 samples, 5.3 (50)	Other Canadian, 14 samples, 2.2 (38, 40)
Iodimetric peroxide, ml. of 0.002 N thiosulphate per gm.	1.6	0.0–14.2	4 samples, 1.5 (28) 6 samples, 0.7 (52) Recommended, 2.5 ml. max. (3)	—
Free fatty acid, as % oleic	0.4	0.1–0.9	5 samples, 0.20 (52) 5 samples, 0.29 (28) 3 samples, 0.33 (57) 4 samples, 0.35 (28) Recommended, 1% max. (3)	General, 0.2–0.7 (22, p. 136)

Expressed as % of total acids.

Magidman et al. performed a quantitative comparison of a commercially rendered lard, a laboratory rendered lard, and a solvent extracted lard representing the same batch of pig tissue. The composition was determined by a preliminary separation of the methyl esters on a silicic acid column followed by gas-liquid chromatography of the fractions containing a known added amount of reference compound. The results are presented in Table 3 (231).

TABLE 3

Composition of Three Lards from GLC Analysis on Total Esters and from Fractions Obtained by Silicic Acid Separation

Component	Commercial lard		Lab. rendered lard		Solv. extracted lard	
	Total[a]	Fraction[b]	Total[a]	Fraction[b]	Total[a]	Fraction[b]
	%	%	%	%	%	%
10:0	0.04	0.01	0.05	0.02	0.04	0.01
12:0	0.07	0.04	0.06	0.05	0.06	0.05
14:0	1.17	1.09	1.17	1.11	1.24	1.06
14:1	0.06	0.02	0.03	.....	0.04	0.01
15:0		0.02		0.02		0.03
16:0	26.4	25.7	26.2	24.8	27.0	27.6
16:1	2.64	2.20	2.16	1.87	2.04	1.74
17:0		0.45		0.21		0.40
17:1	0.28	0.22	0.20	0.29	0.21	0.10
18:0 br	.....	trace	.....	trace	.....	trace
18:0	10.2	12.0	12.5	13.3	11.6	14.4
18:1	47.3	45.2	46.4	45.1	46.5	42.0
18:2	9.52	10.4	9.05	10.6	8.98	9.93
conj. 18:2 *c,t*[c]	.....	0.18	.....	0.09	.....	0.09
conj. 18:2 *t,t*[c]	.....	0.08	.....	0.04	.....	0.04
18:3	0.52	0.34	0.49	0.44	0.56	0.42
19:0	.....	0.04	.....	0.03	.....	0.04
19:1	.....	0.12	.....	0.07	.....	0.08
20:0	0.37	0.18	0.32	0.17	0.35	0.20
20:1	0.93	0.72	0.87	0.87	0.93	0.89
20:2	0.37	0.37	0.40	0.45	0.39	0.38
20:3	trace	0.11	trace	0.07	trace	0.09
20:4	0.46	0.20	0.20	0.20	0.15	0.21
20:5	.....	0.09	.....	0.05	.....	0.09
22:0	.....	trace	.....	.....	.....	0.01
22:2	.....	0.02	.....	.....	.....	.....
22:4	.....	0.05	.....	0.05	.....	0.05
22:5	.....	0.04	.....	0.03	.....	0.02
Unknown	.....	0.03	.....	0.02	.....	0.03

[a] Calculated from a chromatogram of the total lard methyl esters.
[b] Calculated from chromatograms of fractions obtained by silicic acid column separation of lard methyl esters and use of internal standard.
[c] *c,t* and *t,t* = *cis,trans* and *trans,trans*.

In the analysis of lard by gas-liquid chromatography, O'Connor and Herb obtained the results presented in Table 4 (265).

TABLE 4

Lard (and Rendered Pork Fat)[a,b]

Acids	Carbon atoms	Range
Saturated	Less than 14	<1.0
Myristic	14:0	0.5– 2.5
Palmitic	16:0	20 –32
Stearic	18:0	5.0–18
Arachidic	20:0	<1.0
Behenic	22:0	<0.5
Lignoceric	24:0	....
Palmitoleic	16:1	2.0– 4.0
Oleic	18:1	35 –60
Linoleic	18:2	8.0–15
Linolenic	18:3	<2.0
Eicosenoic	20:1	<1.0

[a] Synonym: none.
[b] Fatty acid composition by gas chromatography (wt % of methyl esters).

Upon analysis of lard by gas-liquid chromatography, French obtained the following percentage values for the constituent fatty acids: palmitic 22.8; hexadecenoic 2.9; stearic 18.5; oleic 42.6; linoleic 10.8; linolenic 0.6; arachidic 0.4. Analysis by ultraviolet spectrophotometry produced somewhat different results: oleic 61.3; linoleic 2.5; linolenic 1.1 (104).

VI. Description

A. General Characteristics

Soft, white unctuous mass; slight characteristic odor, bland taste.

B. Physical Properties

Density 0.917; mp 36.42 degrees. Insoluble in water; very slightly soluble in alcohol, freely in benzene, chloroform, ether, carbon disulfide, petroleum ether. Saponification number 195-203. Iodine number 46-70.

C. Stability

Keep cool and in tight containers.

VII. Analytical Methods

Bartlett and Mahon developed an analytical technique whereby an unknown fat or oil may be identified, at least within a group, by differential infrared spectroscopy with olive oil as reference and positive identification made by the spectrum of the unknown with an oil of the group as reference (426).

VIII. Occurrence

A. Plants

None

B. Animals

Lard is purified internal fat from the abdomen of the hog.

C. Synthetics

None

D. Natural Inorganic Sources

None

LARD OIL

Chemical Information

I. Nomenclature

A. Common Names

1. Lard Oil

B. Chemical Names

None

C. Trade Names

None

D. Chemical Abstracts Registry Number

MX8016-28-2

II. Empirical Formula

Not Applicable

III. Structural Formula

Not Applicable

IV. Molecular Weight

Not Applicable

V. Specifications

A. Chemical

See Lard

VI. Description

A. General Characteristics

Colorless or pale yellow liquid.

B. Physical Properties

Density 0.905-0.915. Solidif -2 degrees to +4 degrees. Index of refraction (20 degrees, D) 1.470-1.472. Sapon No. 195-197. Iodine No. 56-74. Acid No. 1.5-2.5. Insoluble in water or cold alcohol; freely soluble in benzene.

C. Stability

No Information Available

VII. Analytical Methods

See Lard

VIII. Occurrence

A. Plants

None

B. Animals

Lard oil is expressed from the fat of swine.

C. Synthetics

None

D. Natural Inorganic Sources

None

Biological Data

I. Acute Toxicity

No Information Available

II. Short-Term Studies

Rats

In a six-week feeding study using rats, Thomasson compared the biological value of lard to that of summer butterfat. The substances were administered to groups of 10-12, 21-day-old Wistar rats at 7 different dietary levels ranging from 10 to 73 Cal%. Results of the tests, as presented in Table 1, indicate that the food-efficiency of lard is comparable to that of summer butterfat. Lard feeding had no adverse effect on mortality or other parameters studied (367).

TABLE 1

Mean increase in weight after 1, 3 and 6 weeks (gm)

Cal. %	Lard				Summer Butterfat I				Summer Butterfat II			
	n	1 w	3 w	6 w	n	1 w	3 w	6 w	n	1 w	3 w	6 w
10	12	24	99	195	12	27	107	206	12	25	100	196
20	10	22	100	199	...	...	...	...	...	...	...	...
30	12	25	109	216	12	26	109	218	12	25	104	211
40	12	25	107	211	12	27	108	211	12	25	105	206
50	12	25	105	218	12	25	111	220	12	24	105	218
60	12	24	104	220	12	26	110	211	12	24	103	217
73	12	23	105	220	12	21	103	216	12	21	99	208
Standard [1]	12	22	97	194	12	28	111	212	12	25	104	205

III. Long-Term Studies

Mice

The effect of a high-lard (24%) diet on the life span of mice was studied by Silberberg and Silberberg. A total of 463 mice, divided into groups as shown in Table 2, were used in the study. The results of the study are presented in Tables 3 and 4. The authors concluded that the high-fat diet significantly increased the mortality and shortened the life span of the males, but not the females, of the C57 strain. Males of the DBA strain were similarly affected but to a much lesser degree (498).

TABLE 2. DISTRIBUTION OF ANIMALS AS TO SEX, STRAIN AND DIET FED

	Strain C57BL				Strain DBA	
	Stock Diet		High-Fat Diet		Stock Diet, Males	High-Fat Diet, Males
	Males	Females	Males	Females		
Total no. of animals observed	100	76	105	54	74	54
No. of animals killed in samples, as scheduled	46	10	50	19	0	0
No. of animals dead or killed because of poor condition and included in this report	54	57	55	35	74	54

TABLE 3. MORTAILITY OF MICE OF VARIOUS GROUPS AT 100 DAYS' INTERVALS

Age	C57♂ Stock Diet, 54 Animals		C57♂ High Fat Diet, 55 Animals		C57♀ Stock Diet, 57 Animals		C57♀ High Fat Diet, 35 Animals		DBA♂ Stock Diet, 74 Animals		DBA♂ High Fat Diet, 54 Animals	
	Mortality	Mean age (range)	Mortality	Mean age (range)	Mortality	Mean age (range)	Mortality	Mean age (range)	Mortality	Mean age (range)	Mortality	Mean age (range)
days	%	*days*	%	*days*	%	*days*	%	*days*	%	*days*	%	*days*
300	2	228							9	288	18	266
400	2	225	18	369	7	381	6	304	31	332	48	323
500	11	409	36	413	26	441	20	431	53	380	76	362
600	28	427	58	461	44	485	40	402	90	452	96	406
700	67	588	91	528	79	548	80	569	99	470	100	416 (210–656)
800	85	617	100	546 (301–730)	100	586 (304–735)	97	601	100	474 (269–717)		
900	100	653 (228–874)					100	606 (392–803)				

TABLE 4. AGES AT WHICH 25, 50, 75, AND 100% OF THE ANIMALS WERE DEAD

Animals Dead	C57♂ Stock Diet		C57♂ High Fat Diet		C57♀ Stock Diet		C57♀ High Fat Diet		DBA♂ Stock Diet		DBA♂ High Fat Diet	
	Max. age	Mean age	Max. age	Mean age	Max. age	Mean age	Max. age	Mean age	Max. age	Mean age	Max. age	Mean age
%	*days*		*days*		*days*		*days*		*days*		*days*	
25	557	481	438	382	486	437	547	456	355	321	311	279
50	664	559	541	447	607	501	621	510	472	373	415	325
75	747	603	637	490	661	542	672	505	551	427	472	308
100	874	653	730	546	735	586	803	600	717	474	656	416

To further study the effect of a high-fat (25% lard) on the life span of mice, Silberberg and Silberberg maintained a total of 441 male mice of the C57BL strain on the regimens outlined in Table 5. The results of the tests are shown in Tables 6 and 7. It was found that feeding a high fat diet from the ages of 6 or 12 months on shortened the life span of the mice, but was less effective in this respect than feeding of this ration from one month of age on. The effect of feeding the high fat diet for 5 months at a time depended upon the period of life during which the fat-enriched diet was consumed. Given during the period of growth, the high fat ration did not alter the life span; in young adults it had a beneficial effect, while given to old animals it exerted

a slightly injurious action on the life span. The authors concluded that old mice were more susceptible to the adverse effect on life span than young adult animals (500).

TABLE 5

DISTRIBUTION OF ANIMALS IN THE VARIOUS GROUPS

Stock diet	High fat diet						
		From 1 month through life	From 6 months through life	From 12 months through life	From 1 to 6 months	From 7 to 12 months	From 12 to 17 months
	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Total number of mice	100	76	61	39	65	51	49
Number of mice killed as scheduled	46	19	33	16	42	30	30
Number of mice dead or killed because of sickness and included in this report	54	57	28	23	23	21	19

TABLE 6

MORTALITY OF ANIMALS OF THE VARIOUS GROUPS AT 100 DAYS' INTERVALS

	Stock diet	High fat diet					
	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Age, days	Mortality, %	Mortality, %					
300	2		4				
400	2	18	11		13		10
500	11	36	14	26	17		16
600	28	58	40	43	22	29	32
700	67	91	93	74	48	62	63
800	85	100	100	100	82	71	79
900	100				95	90	100
1000					100	100	

* *Since less than 100 animals were available in each group, only the full percentages are given.*

TABLE 7

AGES AT WHICH 25, 50, 75, AND 100% OF THE ANIMALS WERE DEAD

Mice dead, %	Stock diet		High fat diet											
	Group I		Group II		Group III		Group IV		Group V		Group VI		Group VII	
	Age, days		Age, days											
	Max.	Mean	Max.	Mean	Max.	Mean	Max.	Mean	Max.	Mean	Max.	Mean	Max.	Mean
25	557	481	438	382	540	424	486	433	606	456	584	541	538	458
50	664	559	541	447	663	507	628	491	709	554	656	587	646	524
75	717	603	637	449	696	567	713	557	719	607	817	635	743	584
100	874	653	730	546	701	600	722	593	972	655	993	694	879	643

Rats

Siedler et al. studied the effects of varying levels of dietary protein, carbohydrate and fats on the nutrition of the rat in a 57-week feeding study. Groups of 14 male weanling rats were maintained on the diets shown in Table 8. After the rats had received the test diets for 4 weeks, those fed diets containing 50% of their calories as casein had superior rates of gain and utilization of crude calories as compared with rats fed diets containing either 20 or 80% of their calories as casein. Lard or corn oil substituted at the expense of sucrose increased the caloric efficiency of rats fed the diets containing 50 or 80% of their calories as casein. No apparent differences in average serum cholesterol, average total serum polyunsaturated fatty acids, and average liver cholesterol were noted between the dietary groups through the initial 22 weeks of this study. After 57 weeks, high levels of dietary casein tended to increase serum cholesterol and to decrease liver cholesterol. Substitution of lard at the expense of sucrose tended to increase serum cholesterol at the low and high dietary casein levels, and to increase total liver cholesterol at all dietary casein levels. Substitution of corn oil at the expense of sucrose tended to decrease serum cholesterol at the intermediate and high dietary casein levels and to increase liver cholesterol at all dietary casein levels. The highest liver cholesterol values were obtained in the groups fed corn oil at the lowest dietary casein level. The average total polyunsaturated fatty acids of the serum was increased in the groups fed the high level of casein plus either lard or corn oil. No evidence of abnormal fat deposits was observed in any of the hearts and aortas from these animals (342).

TABLE 8

Composition of experimental rations

Diet no.	Casein		Corn oil[1]		Lard[2]		Sucrose		Basal fat[3]		Salts IV[4]	Vitamin mix[5]	Cal./100 gm[6]
	Weight	Cal.	Weight	Cal.	Weight	Cal.	Weight	Cal.	Weight	Cal.	Weight	Weight	
	%	%	%	%	%	%	%	%	%	%	%	%	
1	19.8	20	—	—	—	—	73.2	74	2.7	6	4.0	0.3	396
2	33.4	20	—	—	54.9	74	—	—	4.5	6	6.7	0.5	668
3	33.4	20	54.9	74	—	—	—	—	4.5	6	6.7	0.5	668
4	49.5	50	—	—	—	—	43.5	44	2.7	3	4.0	0.3	396
5	65.3	50	—	—	25.5	44	—	—	3.5	6	5.3	0.4	522
6	65.3	50	25.5	44	—	—	—	—	3.5	6	5.3	0.4	522
7	79.2	80	—	—	—	—	13.9	14	2.6	6	4.0	0.3	396
8	85.8	80	—	—	6.7	14	—	—	2.9	6	4.3	0.3	429
9	85.8	80	6.7	14	—	—	—	—	2.9	6	4.3	0.3	429

[1] Mazola, Corn Products Company, Argo, Illinois.
[2] Unstabilized lard.
[3] A mixture of equal weights of corn oil and lard flakes.
[4] Hegsted et al. ('41).
[5] The following vitamins were added per 100 Cal. of the ration: (in milligrams) thiamine·HCl, 0.154; riboflavin, 0.154; nicotinic acid, 0.513; folic acid, 0.051; Ca pantothenate, 1.026; biotin, 0.002; pyridoxine·HCl, 0.154; choline chloride, 38.7; inositol, 25.6; p-aminobenzoic acid, 7.7; vitamin B_{12} (0.1% trituration), 0.513; menadione, 0.8; vitamin A acetate, 0.26; calciferol, 0.002; and DL-α-tocopherol, 1.28.
[6] Calories were calculated as 4 Cal./gm for casein and sucrose, 9 Cal./gm for lard and corn oil.

IV. Special Studies

Reproduction

The influence of a high-lard diet on the reproductive capacity of rats was studied by Velasco and Pancaldi. Five two-month-old female rats, all from a single litter, were employed in the study. Two of the rats (control 1 and 2) were fed bread, milk, and water ad lib, while the other three (test 1-3) were given, in addition to the control diet, 4-5 g of lard daily. One adult female rat (test 4) was also maintained on the high-lard diet and a single male weighing 105 g was alternately kept for one week with the two control rats and for one week with the rats on the high-fat diet (508).

A few days after initiation of the diet containing fat, the three rats, who had previously eaten eagerly, began to lose their appetite, to become emaciated, and to crouch in a corner of their cage. Furthermore, their snouts were somewhat swollen, the eyelids rather edematous and reddened with thinner eyelashes, the fur rumpled and the external genital organs extensively reddened and swollen. The one male rat on an alternating diet, after three or four days with these rats, displayed the same disturbances, with a conspicuous swelling of the scrotum and testicles and priapism. These disturbances diminished until they disappeared after 9-10 days, to reoccur periodically and in a progressively more attenuated form in the female rats. The authors concluded that the drive of the female rats, as well as their reproductive activity, and also the sexual activity of the male, were not in any way altered since one of the females gave birth to a litter almost at the same time as one of the females in the group on a normal diet (508).

All of the female rats employed gave birth to litters, whereupon the mothers and offspring were sacrificed and autopsied. The data attained is presented in Table 9. While the control rats appeared to be completely normal, the growth of the test rats was somewhat stunted. The livers of the rats on the fat-rich diet were enlarged, of a uniformly yellowish color, diminished in consistency, and with the incision surface fairly moist, shining, and oily. Histological examination of the hepatic cells always showed a more or less accentuated state of adiposis (508).

The litters of the control and test rats exhibited no differences with respect to the number of newborns or the time of birth. However, the newborns of the mothers on the diet rich in pork fat, in contrast to the normal, healthy offspring of the controls, appeared weakened, sluggish in their movements, poorly nourished and rather sickly, sucking with difficulty, of an intensely cyanotic color with a dry and wrinkled skin and were considerably smaller than those of the controls. The newborns from the first and fourth litter, the mothers of which had eaten lard for only approximately two months were in better condition than those in the other two; the mothers of these litters had been eating lard for more than three months before delivery. The livers of the pups of the test rats generally appeared small, yellowish in color, soft in consistency, non-compact, and easily reduced to a pulp. Histological examination of liver sections demonstrated a more or less pronounced state of adiposis. The hepatic cells appeared to be strewn with granules and drops of fat, especially abundant at the periphery of the cytoplasm and in some places

TABLE 9. Results of breeding tests on high-lard diet.

	OFFSPRING				MOTHER		
	No. of Offspring	Date of Birth*	Av. Body Wt. (g)	Av. Liver Wt. (cg)	Initial Body Wt. (g)	Final Rody Wt. (g)	Liver Wt. (g)
Control 1	6	July 26	5.47	27.3	54	130	5.87
Control 2	6	Aug 25	5.96	29.5	53	135	5.90
Test 1	5	July 27	4.90	19.8	51	90	5.87
Test 2	8	Sept. 3	3.40	16	58	100	6.40
Test 3	6	Sept. 8	3.50	17.3	55	98	6.20
Test 4	10	Sept. 10	4.25	23	130	150	8

* The experiment was initiated May 30.

grouped so as to form true and proper adipose vesicles. The nuclei of the same cells frequently appeared to have moved to the periphery and the protoplasm appeared to be reduced (508).

In a further test of the influence of a high-lard diet on the reproductive capacity of rats, Velaso and Pancaldi maintained, throughout the period of pregnancy and lactation (2.5-3.5 months), 5 control rats on a normal diet and 3 test rats on the control diet with the addition of 4-5 g of lard per day. Observations on these rats and the litters they produced confirmed the findings of the previous study. In addition, a diversity was observed in the development. While the control sucklings grew normally, the others almost always displayed a stunted growth, with parched and wrinkled skin, a cyanotic color, and sluggish movements. The controls began to grow fine hair after 10-11 days and opened their eyes after 13-14 days, while the other rats began to grow hair after 13-14 days and opened their eyes after 18-21 days. Some died after a few days of life and the survivors, which were sacrificed after 25-27 days of nursing, proved to have a substantially lower weight than the babies

from rats having been fed a regular diet. While the control babies varied in weight between 35 and 36 g (average weight 34.55 g), the other babies ranged in weight from 20-28 g (average weight 23.26 g). Differences were also observed upon macroscopic and microscopic examination of the liver. The livers of the controls were normal in appearance, color, and consistency, with an average weight of 1.57 g, and did not display an abnormal fat deposit in the hepatic cells. On the other hand, the livers of the suckling babies from females on fat diets, in addition to having a uniformly grayish-yellow color, of limited consistency, with an oily and dull appearance in the area of the incision, and averaging 1.20 g in weight, displayed an accentuated state of adiposis upon histological examination. Granules and droplets of fat appeared to be scattered especially at the periphery of the acinus in the hepatic cells. The suckling babies from one of the litters displayed a cutaneous alteration on the tail, which was identified as a characteristic necrosis upon histological examination (508).

Vinson and Cerecedo reported raising rats for four generations on the purified diets detailed in Table 10. Growth on these diets was excellent throughout the four generations and compared favorably with that of controls on Purina dog chow. However, the mothers on the purified diets invariably lost weight during lactation, frequently to a severe degree, and the number of young they weaned was significantly less than the number weaned on the control diet. The mothers rapidly regained weight when separated from their offspring. In an effort to improve lactation, supplementary lard was supplied to the rats ad libitum. The rats ate the lard ravenously, eating as much as 8 g per day, with no improvement in lactation. In fact, the mothers fed the lard supplement destroyed their young during laction. The authors proposed that the consumption of such large amounts of lard interfered with the ingestion of other food constituents (392).

TABLE 10

Composition of Diets

Component	Diet					
	R-1	*R-2*	*R-3*	*R-4*	*R-5*	*R-6*
Purified Casein (Labco or Smaco)	30	30	30	30	30	30
Salts (Osborne and Mendel)	5	5	5	5	5	5
Ruffex[1a]	2	2	2	2	2	2
Lard	10	—	—	2	5	—
Crisco	—	8	—	8	10	10
Corn oil	—	2	10	—	—	—
Sucrose	53	53	53	53	48	53

Supplements were the same for all diets. They were per kilogram: thiamine, 20 mg.; riboflavin, 20 mg.; pyridoxin, 20 mg.; calcium pantothenate, 40 mg.; choline chloride, 500 mg.; α-tocopherol, 20 mg.; vitamin A and D concentrate, 40 mg.

[1a] α-Cellulose preparation obtained from Eimer and Amend, New York.

Arthritis

Silberberg and Silberberg conducted a long-term study to ascertain the effects of a high-lard diet on the development of articular changes in mice. Two hundred and fifty male mice of the strain C57 black were divided into two groups and maintained throughout life on either a stock diet containing 5% fat or the stock diet supplemented with 25% lard. In the mice given the lard supplement articular aging was hastened as compared with that of controls fed the stock diet (see Table 11 and 12). Under the influence of the fat-enriched diet the incidence of osteoarthritis was increased two-fold and the onset of the disease significantly accelerated. As the incidence of osteoarthritis increased, that of simple age changes decreased. A number of mice remained free of articular changes even in old age and even if fed the fat-enriched diet; the number was approximately the same among the test and control animals. As shown in Table 13, the mice fed the high-lard diet were considerably heavier than the controls. The relationship between body weight and the incidence and severity of articular changes was studied by the authors (see Table 14) and they concluded that under the conditions of the experiment the overweight associated with the consumption of the high fat diet played only a minor role, if any, in the pathogenesis of degenerative joint disease (496).

TABLE 11—*The Incidence of Articular Lesions in Various Age Groups*

Age, Mo.	Experimental Group	Mice	Mean Age, Mo.	Percentage of Mice Showing Stated Change: No Change	Age Change: Slight	Age Change: Advanced	Osteoarthritis
9-12	Control	36	10.2	22.2	58.4	11.1	8.3
	Test	33	10.9	24.2	33.3	15.2	27.3
13-18	Control	34	16.8	11.8	40.9	5.9	32.4
	Test	51	16.3	2.0	15.7	13.7	68.6
19-24	Control	36	22.2	5.6	27.7	11.1	55.6
	Test	27	22.0	7.4	0.0	11.1	81.5
25-30	Control	7	27.1	14.3	57.1	0.0	28.6
	Test	0	....	....	....	....	....
9-30 combined	Control	113	17.9	13.5	46.0	8.8	31.9
	Test	111	16.1	9.9	17.1	13.5	59.5

TABLE 12—*The Relationship Between the Articular Findings and the Mean Age of the Animals*

Experimental Group	Mice	Mice Showing No Change: No.	% of Total	Age, Mo.	Mice Showing Age Change: No.	% of Total	Age, Mo.	Osteoarthritis: No.	% of Total	Age, Mo.
Control	113	15	13.3	14.0	62	54.8	16.2	36	31.9	20.2
Test	111	11	9.7	14.0	34	30.6	15.7	66	59.5	17.8

TABLE 13 *Monthly Mean Weights of the Control and Test Animals, with Maximum and Minimum Deviations*

Age, Mo.	Control Animals: Mean, Gm.	Control Animals: Deviation, Gm. Maximum	Control Animals: Deviation, Gm. Minimum	Test Animals: Mean, Gm.	Test Animals: Deviation, Gm. Maximum	Test Animals: Deviation, Gm. Minimum
9	29.0	4.0	2.0	34.6	5.4	3.6
10	30.1	2.9	3.1	35.4	10.6	2.4
11	30.2	2.8	1.2	36.3	10.7	4.3
12	31.1	3.9	2.1	34.3	13.7	4.3
13	31.3	3.7	6.3	36.0	13.0	6.0
14	32.2	3.8	1.2	37.0	22.0	8.0
15	32.3	0.7	5.3	36.1	28.9	5.1
16	31.0	5.0	3.0	35.4	12.6	6.4
17	31.4	3.6	3.4	36.0	18.0	8.0
18	32.2	2.8	1.2	36.8	22.2	9.8
19	31.1	3.9	3.1	35.0	10.0	8.0
20	31.4	3.6	3.4	35.1	12.9	8.1
21	31.4	8.6	3.4	36.2	14.8	4.2
22	31.1	2.9	2.1	40.1	4.9	5.1
23	29.4	1.6	3.4	31.1	0.9	1.1
24	29.3	2.7	2.3	....	....	...
25	26.0	1.0	1.0	....	....	...
26	25.4	0.6	0.4	....	....	...
27	25.0	2.0	1.0	....	....	...
28	26.0	0.5	0.5	....	....	...
29	25.0	1.0	1.0	....	....	...
30	25.0	...	...	....	....	...

TABLE 14 *Findings in the Joints and Their Relation to the Mean Weight of the Animals*

Age, Mo.	Experimental Group	Mice	Mice Showing No Change: Mean Wt., Gm.	Mice Showing No Change: Deviation, Gm. Maximum	Mice Showing No Change: Deviation, Gm. Minimum	Mice Showing Age Change: Mean Wt., Gm.	Mice Showing Age Change: Deviation, Gm. Maximum	Mice Showing Age Change: Deviation, Gm. Minimum	Mice Showing Osteoarthritis: Mean Wt., Gm.	Mice Showing Osteoarthritis: Deviation, Gm. Maximum	Mice Showing Osteoarthritis: Deviation, Gm. Minimum
9-12	Control	31	29.3	0.7	2.[illegible]	30.0	2.0	4.0	33.0	3.0	4.0
	Test	29	33.8	8.2	6.8	35.7	5.3	3.7	40.6	22.4	19.6
13-15	Control	32	33.3	1.7	4.[illegible]	32.9	6.1	2.9	34.7	4.3	3.7
	Test	38	32.5	0.5	0.5	35.6	5.4	7.6	41.2	23.8	9.2
9-18 combined	Control	63	31.6	3.4	3.[illegible]	31.3	7.7	3.3	34.3	4.7	5.3
	Test	67	34.1	7.9	7.1	35.6	5.1	3.6	41.3	23.8	11.2

In a later study, Silberberg, et al. fed 3 groups of mice (male C57BL Jax6), for 18 months, either a stock diet, a diet enriched with 25% lard, or a diet enriched with 23% lard and 3% linoleic acid. As in the previous studies, the mice given the high-lard diet exhibited a much higher incidence of osteoarthrosis than the control animals (see Table 15). The linoleic acid supplement was observed to partly reverse the injurious effect of the high-lard diet. It was noted that the mice of the group fed the high-lard diet showed the fastest and most marked growth, reaching a maximum weight at 12 months of age, but that they exhibited a significant reduction in weight thereafter (see Table 16) (344).

Table 15

Showing the Articular Findings in Stock-Fed and Test Animals

Diet	No. of mice	Mean age at death months	No change No.	No change %	Aging changes slight No.	Aging changes slight %	Aging changes advanced No.	Aging changes advanced %	Osteoarthrosis No.	Osteoarthrosis %
Stock	30	18.0	6	20	7	23	9	30	8	27
Lard	33	17.8	0	0	2	6	6	18	25	76
Lard + linoleic acid	35	17.7	0	0	10	29	11	31	14	40

Significance of differences: Lard: Stock diet $p < 0.001$. Lard: Lard + linoleic acid $p < 0.01$.

Table 16

Showing Mean Weights and Their Ranges of Stock-Fed and Test Animals

Diet	No. of mice	6 months	12 months	15 months	18 months
Stock	30	26.6 (25–28)	31.1 (29–34)	32.3 (23–34)	32.2 (27–33)
Lard	33	29.8 (28–34)	36.4 (31–49)	35.2 (31–47)	26.7 (20–34)
Lard + Linoleic acid	35	29.5 (26–32)	33.3 (20–45)	33.4 (17–46)	31.0 (18–44)

A comparison of the effects of adding 25% lard or 25% cottonseed oil supplements to the regular diet of male C57BL mice was made by Silberberg and Silberberg. As observed in their earlier studies the high-lard diet accelerated articular aging and accentuated the evolution of osteoarthritis. Cottonseed oil, however, produced no adverse effects (343).

Since the female skeleton develops more rapidly and responds to hormonal stimulation less conspicuously than the male skeleton, Silberberg and Silberberg compared the skeletal effects of the high fat diet in both sexes. Twenty-six virgin females of strain C57 black maintained on a 25% lard diet for up to 5 months showed better gain in weight and, histologically, faster epiphyseal development and earlier onset of articular age changes than controls fed the stock diet. However, the differences between the 2 groups of females were less pronounced than those occurring in corresponding groups of males (497).

In order to analyze the relationship between the rate of physiologic development and aging and the skeletal response to a high-fat diet, Silberberg and Silberberg performed feeding studies on mice of the rapidly developing dba strain. The mice were maintained for life on a stock diet with or without a supplement of 25% lard. The results shown in Table 17 indicate that in mice of strain dba, skeletal growth and aging were only

slightly hastened and the incidence of articular lesions was unaffected by feeding a fat-enriched diet. The mice given the fat-enriched diet gained only slightly more weight - about 8% - than the controls, and showed hardly any increase in fat deposits. When compared to the results of the previous study, in which mice of the more slowly developing strain C57 black were used, it appears that the rate of physiologic development of mice significantly effects their response to a high-fat diet - the slower the development the greater the response (499).

TABLE 17

SHOWING THE INCIDENCES OF THE ARTICULAR FINDINGS IN ANIMALS OF THE DIFFERENT AGE GROUPS

Age group months		Total No. of mice	Mean age at death months	No Change No. of mice	No Change %	Sight Age Change No. of mice	Sight Age Change %	Advanced Age Change No. of mice	Advanced Age Change %	Osteoarthritis No. of mice	Osteoarthritis %
7-12	Controls	30	9.4	11	36.7	11	36.7	5	16.6	3	10.0
	Tests	25	9.3	3	12.0	14	56.0	5	20.0	3	12.0
13-18	Controls	40	17.6	2	5.0	20	50.0	5	12.5	13	32.5
	Tests	33	16.1	2	6.1	17	51.5	3	9.1	11	33.3
19-21	Controls	10	19.7	1	10.0	5	50.0	2	20.0	2	20.0
	Tests	5	20.4	0	0.0	2	40.0	1	20.0	2	40.0
Irrespective of age	Controls	80	14.5	14	17.5	36	45.0	12	15.0	18	22.5
	Tests	63	14.0	5	7.9	33	52.4	9	14.3	16	25.4

Sokoloff and Mickelsen reported that although male DBA/2JN mice maintained on diets containing 25% lard or cottonseed oil became significantly heavier than control mice on a stock diet no deleterious effects on the development of degenerative joint disease were demonstrable (see Table 18) (502).

TABLE 18

Summary of observations (exps. 1 and 2)

	Diets, exp. 1		
	Stock	Lard	Cottonseed oil
Peak body weight, g [1]	33.6 ± 0.4 [2] (20) [3]	48.2 ± 1.5 (20)	42.8 ± 1.5 (20)
Final body weight, g	33.0 ± 0.5 (20)	42.9 ± 2.2 (20)	39.9 ± 1.5 (20)
Epididymal pad weight, g	1.07 ± 0.08 (20)	3.24 ± 0.34 (20)	3.00 ± 0.27 (20)
Femur length, mm	15.26 ± 0.04 (20)	15.37 ± 0.04 (20)	15.44 ± 0.04 (20)
Osteoarthritis mean score [4]	0.41 (40)	0.48 (40)	0.21 (40)
Osteoarthritis ridit [5]	0.500 (40)	0.519 ± 0.041 (40)	0.424 ± 0.032 (40)
Survivors	24/24	20/26	23/26

[1] Computed from peak weight of each animal rather than peak month value as in figures 1 and 2.

[2] Mean ± SE.

[3] The numbers in parentheses represent the number of measurements tested.

[4] Based on a severity scale of 0 to 1 (5), recorded for each knee separately.

[5] Because the arthritis scores are discontinuous, the ridit method of Bross (9), as previously adapted (5), has been employed for statistical evaluation of the joint disease. The stock group has been chosen as the identifiable distribution in each experiment; no confidence limits are attached to it.

Foreign-Body Reaction

In a study by Herting and Crain, an unspecified number of weanling male and female Holtzman rats were maintained on a diet containing 50% fully hydrogenated lard while a control group was given 50% untreated lard. Rats were sacrificed at 8-week intervals and subjected to gross examination. Portions of perigastric, perirenal, periadrenal, perigonadal, and subcutaneous fat were removed from each rat and examined microscopically. Gross examination of the viscera and body cavities revealed only a general fullness of the gastrointestinal tract with creamy colored food and an occasional mottled appearance of fat depots in the animals fed the hydrogenated lard. Microscopic examination of the excised fat showed an unusual foreign-body type reaction in fat from the rats fed hydrogenated lard. The lesion consisted of a multinucleated cellular reaction usually located within the fat cell membrane with nuclei arranged evenly about the periphery of the fat cell or in clumps. In many instances, the material which occupied the center of the fat cell, appeared to have been partially dissolved, leaving delicate slit-like spaces which were radially

oriented and suggestive of fatty acid crystals. The reactive process usually involved 2 or more adjacent fat cells and was separated from similar areas by uninvolved fat cells. No acute inflammatory reaction, evidence of recent hemorrhage, or birefringent material were associated with the fat reaction. The foreign-body reaction occurred within 16 weeks in rats fed hydrogenated lard, but was not observed in the control rats (449).

Carcinogenicity

To test the carcinogenicity of lard, Barry and Cook injected lard s.c. into the groin of rats and mice (at first 1 ml in rats and 0.5 ml in mice weekly; later 3 ml in rats and 1 ml in mice at longer intervals, regulated by the rate of disappearance of the substance). As presented in Table 19, of 20 mice injected none developed tumors, while of 10 rats injected 2 produced tumor-like masses of tissue at the injection site. This tissue was found to consist largely of necrotic matter and granulation tissue, with some spindle-celled areas in which the cells were mostly much less closely packed than those in tumors following the injection of 1:2:5:6-dibenzanthracene. According to the authors the tumor-like tissue produced by lard injections showed none of the characteristics of malignancy (22).

Table 19: *Subcutaneous Injection of Hydrocarbons, and of Lard*

Substance injected	Animals R = Rats M = Mice	Number of Animals in which Tumours Appeared	Day of Death of Tumour-bearing Animals		Transplantable Tumours		Remarks
			Range	Average	Reference Number of Primary Tumour	Result of Graft	
Lard	10R	2	385, 462		R113 R141	Negative Negative	L.R.D. day 721
	20M	0	—	—	—	—	L.M.D. day 581

gen. = generation. L.R.D. = last rat died. L.M.D. = last mouse died.

As part of an experiment testing for carcinogenicity, Burrows et al. subjected 134 rats to subcutaneous injections of lard (see Table 20). Weekly injections of 1 ml were given until a bulky collection of lard was present under the skin, after which the intervals between the injections were increased, being regulated by the size of the subcutaneous collection. Among those rats given lard, seven spindle-celled tumors arose at the site of injection. It was pointed out that the tumors required a long period for their appearance, none developing during the first year. The induced tumors consisted of spindle cells, were firm to the touch, grew to a large size, eventually invaded surrounding structures, underwent central necrosis, ulcerated through the skin and killed the hosts. No metastases were observed and the tumors when grafted into other rats failed to grow in every case. The workers concluded that the observed tumors may represent a low grade of malignant neoplasia (49).

TABLE 20

Subcutaneous injections of lard and olive oil in rats.

No. of expt.	Duration in weeks.	Number of rats.	survived four months.	Material injected.	Site of injection.	Spindle-celled tumour at site of injection.	Period in weeks from first injection to death of tumour-bearing animals.	Other treatment.
1	146	20	17	Lard	Groin	2	79, 146	None
2	99	7	6	Lard	Groin	0	...	None
3	121	10	8	Lard	Groin	1	121	None
4	81	10	6	X-radiated lard	Groin	0	...	None
5	69	10	5	Lard	Groin	1	98	None
6	89	10	7	Lard + 5 per cent. oleic acid	Groin	1	79	None
7	72	7	7	Lard + theelin (Parke Davis and Company)	Groin	1	56	None
8	53	10	7	Lard	Nape	1	53	None
9*	105	20	16	Lard	Nape	0	...	Emulsified olive oil into peritoneum
10	94	30	23	Lard	Nape	0	...	1 : 2 : 5 : 6-dibenzanthracene into peritoneum
11	80	8	6	Olive oil	Groin	0	...	None
12	71	8	6	Olive oil + œstrone (0·03 per cent.)	Groin	0	...	None
13	48	3	2	Olive oil + œstrone (0·03 per cent.)	Groin	0	...	None
14	106	40	28	Olive oil + arsenious acid †	Groin	1	97	None
Totals	...	193	144	...	...	8	...	...

* See also table V.

† Contained 0·0173 per cent. $NaAsO_2$, which gives a concentration of As (0·01 per cent.) of the order of that found in the hyperkeratotic skin of persons liable to arsenic cancer.

Unsuccessful attempts were made to graft the tumours of expts. 3, 6, 7, 8 and 14 into young rats, ten of which were used for each attempt.

A polypus of the uterus was present in one of the tumour-bearing rats of expt. 1. In expt. 12, in which the injected olive oil contained œstrone, mammary tumours appeared in two of the rats (31st and 64th weeks) and a sarcoma of the liver adjacent to a *cysticercus* cyst in another.

A *cysticercus* sarcoma was present also in one of the rats of expt. 14. In expt. 13, in which œstrone was present in the injected oil, lymphosarcomatosis appeared in one of the rats. In expt. 10, in which 1 : 2 : 5 : 6-dibenzanthracene was injected into the peritoneum, 23 of the animals survived for four months or longer and peritoneal tumours appeared in 12 (52 per cent.) of these.

In further testing for carcinogenicity, repeated intraperitoneal injections of lard or lard plus 5% oleic acid were made by Burrows et al. in 20 mice and 20 rats. At first the injections were given once a week, and later at gradually lengthening intervals. The tests were continued for up to 40 and 124 weeks for the mice and rats, respectively. No injection site tumors were observed in any of the test animals (49).

As controls in an experiment conducted by Andervont, 190 mice of 5 pure strains and one mixed stock were given initial subcutaneous injections of 0.25 ml of lard, followed 2 weeks later by injections of 0.25 ml, and finally after another 2 weeks by injections of 0.50 ml. The animals were observed for 27 weeks after the initial injection, whereupon they were autopsied and examined macroscopically for the presence of tumors. In no case was the development of a tumor observed (423).

Atherosclerosis

There has been a great deal of controversy in the past, and in the present, on the role of saturated and unsaturated fats in the synthesis of cholesterol, serum lipid and cholesterol levels and the evolution of atherosclerosis. As of yet this controversy remains unresolved and it is not within the scope of this monograph to try to resolve it. Brief mention is given to the small number of available reports which deal with lard per se.

In their studies on male Long-Evans rats, Wilson and Siperstein found that dietary supplements of 30% lard had no effect on either the excretion of total cholesterol-C^{14} end producteds or of cholesterol itself into bile. Furthermore, the feeding of lard, at either the 10% or 30% level, had no effect on the rate of conversion of acetate or mevalonate to cholesterol (404).

Kritchevsky and Tepper maintained male rabbits of the Dutch belted strain (av. starting weight 1.7 kg) for two months on a diet consisting of rabbit chow augmented with 2% cholesterol suspended in 6% fat. By this means it was shown that lard is considerably more atherogenic than corn oil (201).

Downie et al. reported that when given to swine diets rich in lard accelerate the development of atherosclerosis, enhance blood coagulation and thrombus formation and raise the serum lipid level (80).

Linazasoro et al. reported that the feeding of a 15% lard diet to rats for 3 days increased the liver's capacity for incorporating acetate carbon to cholesterol. When lard was compared to corn oil, Wesson oil and a hydrogenated vegetable oil it was found these fats were of about equal value in stimulating hepatic cholesterogenesis. No effect on the lipid or glycogen content of the liver or on the lipid content of the plasma was observed with any of these fats (217).

When dogs were maintained on a high-lard (30%) diet, Glomset and Bollman were able to discern a moderate sustained depression of plasma lipid fractions. This depression was greater when the quantity of food fed was diminished (112).

When fed to pigs, diets containing 25% lard were found by Mustard et al. to significantly raise the platelet adhesive index, to increase serum cholesterol, phospholipids and lipoprotein, and to increase the weight of deposit formed in an extracorporeal shunt (254).

Biochemical Aspects

I. Breakdown

In the introduction to a report on the cumulative and chronic effects of heated fats, Poling et al. made the following remarks (298).

> "Edible fats undergo chemical and physical changes when exposed to heat/or oxygen or both and, if the treatment is severe enough, some of the products may be harmful to experimental animals when fed under appropriate conditions. The nature and extent of the changes have been investigatived extensively during the last few years, yet because of the complexity of the mixtures, there is still much to be learned regarding the composition and the biological effects of heated or oxidized fats. Three general types of chemical changes occur: oxidation, cleavage to smaller compounds and polymerization.
>
> There is considerable evidence that the treatments to which edible fats may be subjected during practical home or institutional use do not cause the formation of deleterious substances in amounts sufficient to impair health. On the other hand, several teams of scientists have observed adverse biological effects when fats which had been severaly heated or extensively oxidized were fed or injected."

Alfin-Stater et al. likewise stated that the laboratory heat-treatment of fats used in studying the effects of feeding heated fats is unrealistically drastic (5).

In the experiments reported by Poling et al. (298) and Alfin Stater et al. (5) heated lard was fed to rats for long periods without the production of adverse effects.

Poling et al. compared the nutritional value of lard which had been heated at 182 degrees C for 120 hours to that of unheated lard by feeding both substances to rats as 20% of nutritionally adequate diets for 928 days. Differences in the responses of rats fed diets containing the unheated and heated lard were generally small with respect to rates of gain, 12th week and adult weights, efficiencies of utilization of absorbed energy, incidences of grossly detectable diseases and longevities. There were no indications that the feeding of the heated fat had shortened survival times in comparison with the unheated fat. Food efficiency was slightly lower with the heat lard diet because of decreased digestibility of the lard. Tumor incidence was unaffected by the consumption of heated lard (298).

In a study by Alfin-Slater et al., lard was heated in vacuo for 60 and 70 minutes at 400 and 610 degrees F, respectively, and was fed to male and female rats at a level of 15% in the diet over a complete life span. Nutritional indices such as growth, including food efficiency

and digestibility of the lard, reproduction and lactation, longevity, and tissue cholesterol and total lipid gave no evidence of impaired nutrition or harmfulness of the diet. There was no evidence of increased tumor incidence in the animals ingesting the heated lard (5).

The following five reports are presented as representative of the studies in which adverse effects have been observed following the feeding of heat-treated lard to rats.

Beck and Peacock reported that rats maintained on an adequate basal diet supplemented with lard which had repeatedly been heated to 200-220 degrees C developed, within a year, signs of avitaminosis-A. Cases of ulceration and papillomatosis of the fore-stomach were observed among those that died. Control animals fed with unheated fats and the same basal diet showed no gross pathological lesions. Extracts of the livers of rats fed, for 2 months, with lard heated under various conditions in addition to the basal diet contained reduced amounts of vitamin A as compared with controls (see Table 1). Further feeding studies showed that the addition of vitamin A to the diet in the form of raw carrot counteracted the anti-vitamin effects of the heated lard (428).

TABLE 1.– *Relative Vitamin A Contents of Rat Livers*

Rat No.	Lard	Vitamin A Content of Liver (Control = 1)
375B 376B	Unheated (control)	1 1
372B 373B	Heated, 220° C. 1 hr.	1 1
363B 366B	Heated, 220° C. 12 hrs.	1/2 1/2
361B 362B	Heated, 327° C. 1 hr.	1/4 1/8

Peacock and Beck reported that rats maintained on (a) adequate diet, (b) restricted diet (bread or potatoes and milk), to which were added lard heated to 220 degrees C or 350 degrees C, developed multiple mesenteric sarcomata in 4 out of 71 animals surviving more than 300 days (286).

To test the effects of oxidized fats (lard and corn oil aerated at 40 degrees C until peroxide values of 100 and 150, respectively were attained) on the reproduction of rats, Kennelly and Quackenbush maintained six groups of weanling Wistar rats on the diet shown in Table 2. The fat portion of the diet was fed either incorporated into the diet or separately by dropper; in the latter case the rats were restrained from food for 3 hours after administration of the fat to minimize the intra-intestinal mixture of the fat with the other dietary components. The reproduction record presented in Table 3 indicates that the consumption of oxidized lard significantly interfered with reproduction when fed with the diet, but not when fed by dropper. This indicates that the oxidized fat exerted no toxic effects itself, but destroyed some essential component of the non-lipid portion of the diet (188).

TABLE 2

Experimental Diet

Casein (5 day alcohol extracted)	18
Salt mixture (McCollum #185)	4
Yeast (Fleischmann's irradiated)	5
Dextrin	56
Celluflour	2
Fat[1]	15
Fresh fat supplement[2]	

[1] Fresh lard, oxidized lard or oxidized corn oil mixed with diet or fed by dropper.
[2] 2 ml. of corn oil containing 100 I.U. of vitamin A was administered weekly to each rat.

TABLE 3

The Effect of Oxidized Fat on Reproduction

Group[1] Rats	Fat	Litters[2]	Rats Born	Rats Weaned	Average Weight at 21 Days (gm.)
1.	Fresh lard[3] (dropper)	9	70	29	36.1
2.	Oxidized lard[3] (dropper)	9	82	27	35.3
3.	Oxidized corn oil[3] (dropper)	7	70	26	35.8
4.	Fresh lard[4]	6	73	28	36.6
5.	Oxidized lard[4]	3	37	18	37.0
6.	Oxidized corn oil[4]	1	14	5	35.4

[1] Each group contained 12 female and 4 male rats.
[2] Twelve matings attempted for each group.
[3] Fed on alternate days in amounts sufficient to make up 15% of total diet consumed.
[4] Incorporated in the diet at a 15% level.

In a study by Morris et al., groups of 20-30 rats were fed, for 21 months, nutritionally adequate diets containing lard that had been heated as shown in Table 4. The control animals (unheated lard) were able to increase in body weight throughout the test period and showed no gross clinical evidence of nutritional deficiency. However, as shown in Figure 1, the animals given heated fat were scarcely able to maintain their body weight and many lost weight during the test period. A progressive type of paralysis having characteristics of vitamin E deficiency in adult rats was observed in some of the animals that were fed the heated-fat diets. The paralysis first involved the forelegs and head and was accompanied by tremors; in the later stages a definite lack of co-ordination in walking was observed. Upon autopsy neither gastric nor hepatic neoplasms were found under the experimental conditions employed. Chronic gastric ulcers appeared in a few rats, and cirrhosis of the liver in many fed the heated-fat diets. Some microscopic lesions were observed in the heart and kidneys (247).

TABLE 4.—*Diets used in experiments with heated lard*

Diet No.	Fat component	Treatment		Proportion of diet
		Temperature of heating	Time of heating	
		°C.	*Minutes*	*Percent*
71	Lard	None	None	50.0
72	do	300	30	50.0
73	do	350	30	35.0
	do	None	None	15.0
74	do	300	120	50.0
75	do	None	None	38.0
	Free fatty acids from unheated lard	None	None	12.0

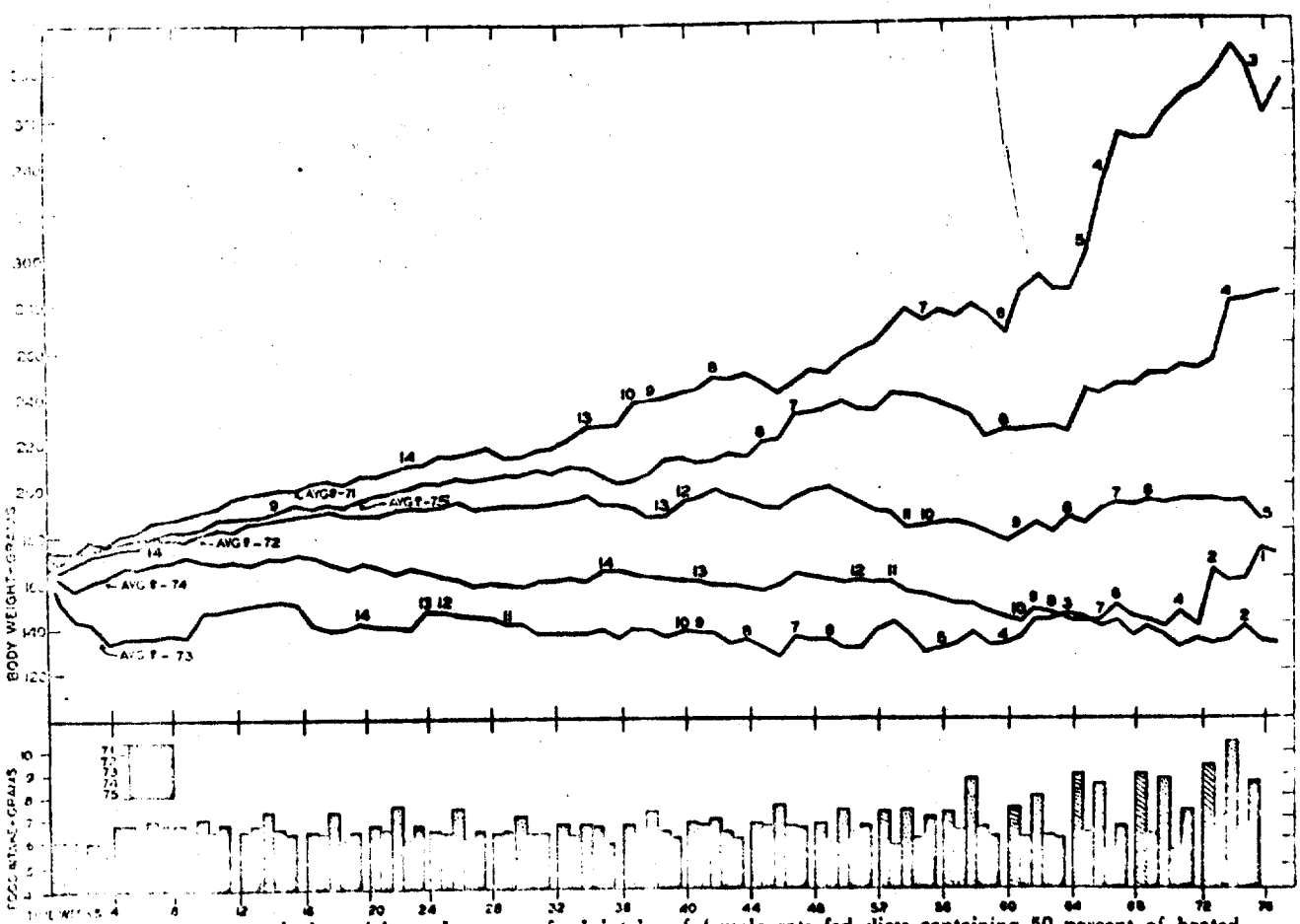


FIGURE 1.—Average body weight and average food intake of female rats fed diets containing 50 percent of heated and unheated lard.

In a feeding test lasting 78 days, Nadeau and Lewis fed weanling rats rations containing 20% fresh lard or lard which had been autoxidized at 50 degrees C to peroxide values of 150 or 300 mEq/kg. When the ration contained only 12% protein significant decreases were observed in growth rates, nitrogen retention, and fresh weights of livers, spleens and seminal vesicles from rats fed rancid lard rations. These differences were of relatively small magnitude and were not observed in preliminary studies when the rations contained 18 or 30% protein or when older rats were used (256).

II. Absorption-Distribution

With the aid of an adiabatic colorimeter, Petersen and Vik-Mo determined the energy content of lard to be 9.424 kcal/g. In a study with young, growing chickens they found the metabolizable energy of lard to be 7.92 kcal/g. The digestibility coefficients determined directly and calculated from the metabolizable energy are 92.7 and 84.0%, respectively (293).

The metabolizable energy value and percent utilization of lard by chicks from 2 to 8 weeks of age were found by Renner and Hill to be 3980 Cal/lb and 93.4%, respectively, when the lard was fed at a level of 20% of the diet. Hydrolysis of the lard to the form of free fatty acids reduced these values to 2730 Cal/lb and 64.8%, respectively (488).

To determine the degree of utilization of lard by chickens of various ages, Renner and Hill fed lard at a level of 17.5% in a basal diet. The results of the test (see Table 5) indicate that the utilization of lard is relatively constant for growing chicks. However, it is somewhat reduced for adult hens as a result of reduced absorbability. The authors noted that in subsequent work the absorbability of lard was not significantly different for chicks and adults (312).

TABLE 5 *Utilization of lard by chickens of various ages*

Age in weeks	Energy utilization: Metabolizable energy		Energy utilization: Percent of gross energy[1]		Absorbability[2]	
	Cal./gm.				%	
2 weeks	8.69		92		91	
	8.55	8.62[3]	91	92	90	90
4 weeks	8.30		88		91	
	8.50	8.40	90	89	92	91
6 weeks	8.46		90		93	
	8.81	8.63	94	92	96	95
8 weeks	8.67		92		92	
	8.72	8.70	93	92	92	92
38 weeks (adult hens)	7.81		83		86	
	7.72	7.76	82	82	85	85

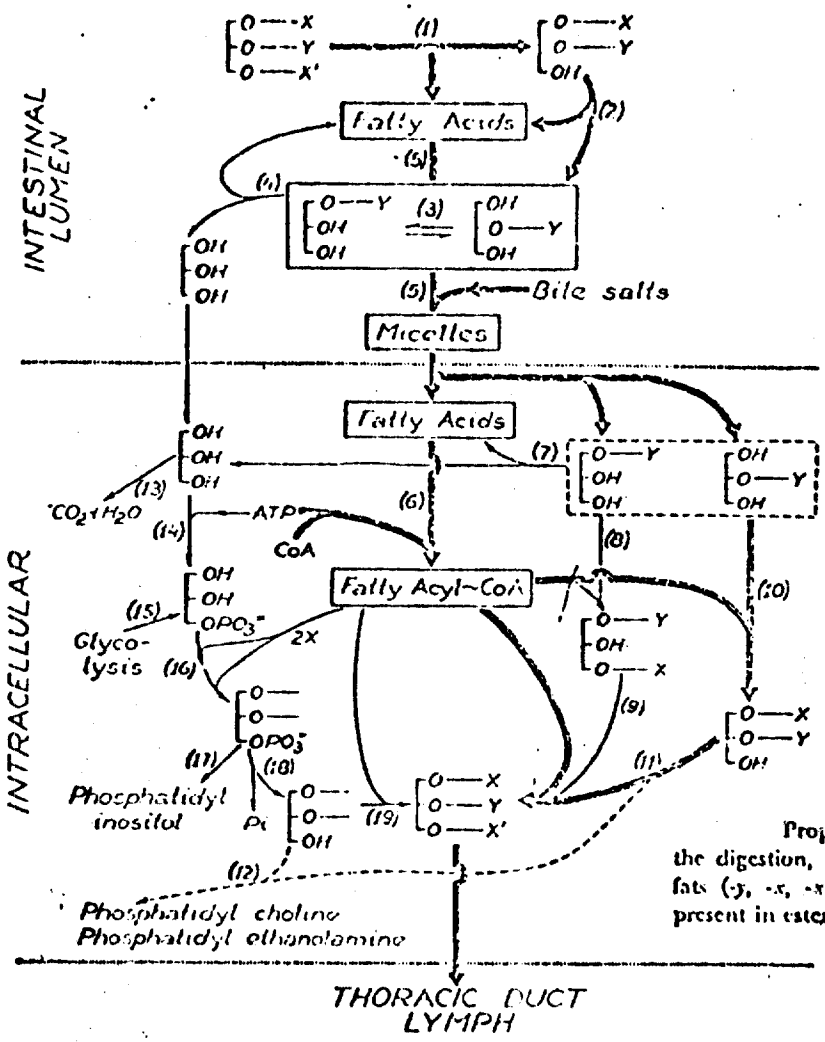
[1] Metabolizable energy as a percentage of gross energy of lard (9.42 Cal./gm.).
[2] Computed from dietary fat and total fecal fat correcting for fecal fat from low fat reference diet.
[3] Underlined values are averages of duplicate lots; individual replicate values are in the respective left columns.

After feeding young pigs a diet containing 10% lard for a period of 9 days, Carlson and Bayley determined the digestibility coefficient of lard to be 81% (54).

Langworthy determined the digestibility of lard through nine 3-day oral tests with human volunteers. When consumed at a level of 50-115 g/day, a value of 97% was obtained (466).

According to Kayden et al. analysis of intestinal lumen contents of man after fat feeding has shown a separation of glycerides into two phases: an oil phase which contains mainly diglycerides and triglycerides and some fatty acid, and a micellar phase which is a combination of bile salts, free fatty acids, and monoglycerides. From the micellar solutions monoglycerides and free fatty acids are absorbed through the intestinal wall, leaving the bile salts within the lumen to form additional micelles. The fate of the free fatty acids within the mucosal cell is determined by their chain length and saturation; those of less than 12 carbons are preferentially absorbed by rats directly into the portal blood, whereas longer chain fatty acids are incorporated mainly into triglycerides, and appear in lymph chylomicrons. Monoglycerides within epithelial cells may be esterified to di- and triglycerides or cleaved to glycerol and free fatty acids. The 2-monoglycerides are esterified especially rapidly to triglycerides, but esterification of 1-monoglycerides lags and these are more likely to undergo hydrolysis particularly if the fatty acids are of short chain length (462).

In a review of the literature of fat absorption, Johnston stated that the mechanism of fat absorption can be summarized as shown in the following figure.



Proposed mechanism involved in the digestion, absorption, and biosynthesis of fats (-y, -x, -x', and —— denote fatty acids present in ester linkages).

The heavy arrows denote the major pathway. The emulsified triglycerides are hydrolyzed by pancreatic lipase, at the 1 and 3 positions, forming monoglycerides and fatty acids which in combination with bile salts form a micellar dispersion (reaction 5). The monoglycerides and fatty acids form micelles which penetrate the lumen membrane by an energy-independent mechanism, presumably by diffusion (reaction 5). The monoglycerides and fatty acids migrate to the endoplasmic reticulum, where the fatty acids are activated to their CoA derivatives (reaction 6) and are resynthesized into triglycerides by the acylation of monoglycerides in a multienzyme complex (reactions 10 and 11) (458).

III. Metabolism and Excretion

Fatty acids play an important role as an energy source. Their oxidation is prominent in higher animals which can store large amounts of neutral fat as a fuel reserve. It is estimated that fatty acid oxidation provides at least half of the oxidative energy in the liver, kidneys, heart muscle and resting skeletal muscle. Fatty acids undergoing oxidation in tissues of higher animals come either from extracellular

fluid or from endogenous intracellular lipids. Vertebrate blood contains considerable amounts of triacylglycols but very small amounts of free fatty acids. The major endogenous source of fatty acids for fuel is storage fat, in the form of fat droplets in the cytoplasm, which consists largely of triacylglycerol. Before the fatty acids can undergo activation and oxidation, the triacylglycerols first must undergo hydrolysis by intracellular lipases to yield free fatty acids and glycerols. Free fatty acids are therefore derived either from the blood stream, in which they are transported bound to serum albumin, or prior hydrolysis of intracellular lipids. They are first activated by esterification with CoA to form acyl CoA esters of the outer mitochondrial membrane. Then the fatty acyl group from CoA is carried across the inner membrane by the carrier molecule carnitine. Finally the fatty acyl group is transferred from carnitine to intramitochondrial CoA through several enzymatic reactions. A two-carbon fragment of the fatty acid is removed as acetyl CoA and two pairs of hydrogen atoms are given to specific acceptors. A long chain fatty acid will go through several spirals. Thus the 16-carbon palmitic acid undergoes a total of 7 such cycles, to yield 8 molecules of acetyl CoA and 14 pairs of hydrogen atoms. The acetyl CoA formed as a product of the fatty acid oxidation will then enter the tricarboxylic acid cycle, which is the final common pathway of oxidation catabolism of all fuel molecules in aerobic cells (468).

IV. Effects on Enzymes and their Biochemical Parameters

No Information Available

V. Drug Interaction

Houssay and Martinez reported that the toxic and diabetogenic action of alloxan increases in rats fed a high-lard (34%) diet. The action of these fat diets was corrected by the addition of methionine, thiouracil, or coconut oil, but there was no modification either by addition of choline or sulfonamide or by increase in the protein proportion. The unfavorable effect of the high-lard diet was observed also on the initiation and evolution of diabetes due to subtotal pancreatectomy (160).

In a study by Kline et al., six groups of 15 rats each were fed 0.06% p-dimethylaminozobenzene for 4 months in synthetic diets as shown in Table 6. The carcinogenicity of p-dimethylaminoazobenzene was considerably higher in the high-fat diets than in the low-fat diet. Dietary lard was found to produce a lower incidence of hepatomas than corn oil (195).

TABLE 6 THE EFFECTS OF THE KIND AND THE LEVEL OF CERTAIN FATS ON THE CARCINOGENICITY OF p DIMETHYLAMINOAZOBENZENE

Group	Diet	Average starting weight, gm.	Average weight at 4 mos., gm.	Average food consumption, gm./rat/day	Survival * at 4 mos.	Hepatomas † at 4 mos.	Hepatomas † at 6 mos.	Negative survivors at 6 mos.	Gross cirrhosis at 4 mos. none	Gross cirrhosis at 4 mos. moderate	Gross cirrhosis at 4 mos. severe
1	Low-fat	162	221	11.0	13/15	1/13	3/13	10	11	2	0
2	5% Corn oil	161	213	9.7	15/15	11/15	14/15	1	1	8	6
3	5% Olive oil	153	236	9.7	15/15	2/15	8/15	7	13	1	1
4	20% Corn oil	162	176	7.3	15/15	15/15		0	0	0	15
5	20% Crisco	151	200	8.4	15/15	7/15	13/15	1	3	7	5
6	20% Lard	156	212	7.8	15/15	9/15	13/15	2	1	9	5

* Survival = Number living over number at start

† Hepatomas = Number with hepatomas over number surviving at 4 months

The tumor-promoting effects of lard filtrate, lard residue, and fractions of both when used as the vehicle for single subcutaneous injections of 3,4-benzpyrene were examined by Leiter and Shear. The specifics and results of the tests are presented in Tables 7-9. Lard residue exercised a striking retardation of tumor genesis by benzpyrene, while lard filtrate promoted rapid tumor production in a high proportion of the test animals. The variations when lard filtrate was the vehicle were apparently a consequence of the different proportions of retarding and promoting substances in the various specimens of lard filtrate employed. Fractionation of both the filtrate and residue of lard by various methods showed the retarding influence to residue in the glycerides which were richest in saturated fatty acids of high molecular weight (212).

TABLE 1.—*Reproducibility of production of subcutaneous tumors with the same sample of lard as vehicle for 3,4-benzpyrene*

Experiment No.	Benzpyrene injected	Mice[1] with tumors (+), and negative survivors (−), after various periods		
		4 months	6 months	9 months
	Milligrams	*Percent*	*Percent*	*Percent*
LARD FILTRATE				
38a	0.1	72+ / 22−	84+ / 6−	86+ / 2−
38b	.1	88+ / 8−	94+ / 2−	94+ / 2−
43	.065	72+ / 28−	84+ / 16−	84+ / 16−
98	.065	62+ / 36−	80+ / 18−	84+ / 8−
LARD RESIDUE				
39a	.1	0+ / 90−	4+ / 78−	6+ / 66−
39b	.1	0+ / 100−	4+ / 94−	10+ / 70−
100	.1	2+ / 98−	4+ / 84−	14+ / 40−
44[2]	.065	0+ / 86−	4+ / 70−	4+ / 62−

[1] 50 mice, equally divided between the sexes, used in each experiment.
[2] For comparison, data obtained in a single experiment with a lower dose are included here.

TABLE 2.—*Variation in production of subcutaneous tumors in mice with 0.1 mg. of 3,4-benzpyrene in different specimens of lard filtrate as vehicle*

Experiment No.	Specimen of filtered lard used	Number of mice[1]	Mice with tumors (+), and negative survivors (−), after various periods		
			4 months	6 months	9 months
			Percent	*Percent*	*Percent*
S-1	A	20	5+ / 95−	25+ / 75−	30+ / 65−
S-2	B	20	85+ / 15−	95+ / 5−	100+ / 0−
20	C	40	30+ / 70−	55+ / 40−	60+ / 35−
38a	D	50	72+ / 22−	84+ / 6−	86+ / 2−
38b	D	50	88+ / 8−	94+ / 2−	94+ / 2−

[1] The mice were equally divided between the sexes, except in experiment S-1 in which only male mice were used (*4, table 1, experiment C, control group*).

TABLE 3.—*Production of subcutaneous tumors in mice with different dose levels of 3,4-benzpyrene in the same specimens of tricaprylin as vehicle*

Experiment No.	Tricaprylin employed	Benzpyrene injected	Number of mice[1]	Mice with tumors (+), and negative survivors (−), after various periods			
				4 months	6 months	9 months	12 months
		Mg.		*Pct.*	*Pct.*	*Pct.*	*Pct.*
L-14	Specimen A	0.1	24	33+ / 67−	67+ / 33−	71+ / 17−	71+ / 0−
L-15	do	.05	23	13+ / 83−	30+ / 66−	47+ / 44−	47+ / 34−
L-23	Specimen B	.2	60	45+ / 55−	68+ / 30−	80+ / 15−	82+ / 3−
L-22	do	.1	60	12+ / 85−	63+ / 33−	75+ / 20−	75+ / 8−
L-21	do	.065	60	3+ / 97−	32+ / 66−	50+ / 37−	53+ / 10−
E-6	do	.05	48	4+ / 96−	21+ / 70−	38+ / 40−	42+ / 20−
E-10	do	.05	50	2+ / 98−	24+ / 70−	24+ / 66−	24+ / 24−
L-26	Specimen C	.2	60	23+ / 73−	45+ / 47−	53+ / 30−	53+ / 3−
L-25	do	.1	60	3+ / 92−	37+ / 55−	47+ / 37−	49+ / 13−
L-24	do	.065	60	3+ / 95−	28+ / 70−	37+ / 50−	43+ / 28−
80	Specimen D	.1	50	28+ / 66−	58+ / 36−	66+ / 16−	...
85	do	.065	50	14+ / 84−	58+ / 38−	60+ / 28−	...

[1] Mice equally divided between the sexes.

VI. Consumer Exposure

The following tables were obtained from Agricultural Statistics (519).

Table 204.—Margarine: Fats and oils used in manufacture, United States, 1949–71

Year	Vegetable oils							Animal fats and oils		Total[1]
	Soybean oil	Cottonseed oil	Peanut oil	Corn oil	Coconut oil	Safflower oil	Other	Lard	Beef fats	
	Million pounds	*Million pounds*	*Million pounds*	*Million pounds*	*Million pounds*	*Million pounds*	*Million pounds*	*Million pounds*	*Million pounds*[2]	*Million pounds*
1949	257	431	(3)	1	(3)	.....	.....	4	7	701
1950	312	418	7	1	.....	.....	2	4	9	764
1951	473	334	16	4	1	.....	2	4	7	851
1952	652	354	3	(3)	.....	.....	.....	5	8	1,046
1953	726	275	2	1	7	.....	14	8	13	1,046
1954	665	397	2	(3)	5	.....	18	7	10	1,108
1955	746	279	2	(3)	6	.....	17	13	9	1,071
1956	752	283	3	1	8	.....	25	31	6	1,111
1957	874	237	3	(3)	5	.....	27	25	9	1,152
1958	1,070	145	4	1	4	.....	21	16	8	1,268
1959	1,094	124	3	20	4	.....	3	36	8	1,291
1960	1,105	136	4	55	4	.....	1	56	6	1,367
1961	1,062	139	3	89	3	.....	11	72	6	1,386
1962	1,058	106	4	99	5	6	37	70	10	1,304
1963	1,049	104	4	136	4	22	36	84	11	1,451
1964	1,139	101	10	150	6	12	5	64	13	1,500
1965	1,112	114	4	161	5	10	14	100	14	1,535
1966	1,294	106	6	157	12	46	1	82	5	1,710
1967	1,249	78	5	176	15	42	4	125	10	1,708
1968	1,240	70	4	179	14	42	3	153	15	1,720
1969	1,332	76	3	172	15	44	3	86	13	1,744
1970	1,410	68	.....	185	10	22	1	90	9	1,794
1971[4]	1,385	63	.....	186	7	19	2	156	10	1,831

[1] Includes 2 million pounds of secondary oils other than vegetable stearine in 1952, 3 million in 1953, 1954, 1955, and 1956, and 2 million in 1957 and 1958.
[2] Less than 500,000 pounds.
[4] Preliminary.

Economic Research Service. Compiled from reports of the U.S. Department of Commerce. Totals computed from unrounded numbers. Data for 1944-48 in *Agricultural Statistics, 1967*, table 207.

Table 206.—Shortening: Fats and oils used in manufacture, United States, 1949–71

Year	Vegetable oils								Animal fats		Vegetable stearine	Glycerides	Total primary and secondary fats and oils
	Cottonseed oil	Soybean oil	Coconut oil	Peanut oil	Corn oil	Palm oil	Safflower oil	Other[1]	Lard	Beef fats			
	Million pounds	*Million pounds*	*Million pounds*	*Million pounds*	*Million pounds*	*Million pounds*	*Million pounds*	*Million pounds*	*Million pounds*	*Million pounds*	*Million pounds*	*Million pounds*	*Million pounds*
1949	532	713	20	12	1	.....	.....	3	118	31	64	.....	1,4[illegible]
1950	549	841	0	12	1	.....	.....	27	177	31	89	.....	1,7[illegible]
1951	335	731	20	21	1	.....	.....	25	200	23	48	.....	1,4[illegible]
1952	388	851	33	6	1	.....	.....	2	232	24	66	.....	1,613
1953	376	913	2	4	1	.....	.....	4	227	46	62	35	1,6[illegible]
1954	640	918	15	5	1	.....	.....	20	142	89	99	39	1,9[illegible]
1955	439	930	4	6	3	.....	.....	15	334	112	99	40	1,9[illegible]
1956	323	782	6	6	2	.....	.....	6	459	141	90	38	1,855
1957	272	796	8	6	4	.....	.....	3	376	226	95	37	1,824
1958	239	1,055	12	5	3	.....	.....	2	318	252	86	39	2,011
1959	320	1,143	20	1	5	.....	.....	5	493	257	(3)	(3)	2,244
1960	365	1,169	10	2	4	.....	.....	3	490	268	.....	.....	2,[illegible]
1961	356	1,160	26	3	14	11	.....	11	530	348	.....	.....	2,459
1962	367	1,362	27	1	7	16	4	3	571	338	.....	.....	2,6[illegible]
1963	330	1,228	19	3	3	14	3	5	594	413	.....	.....	2,[illegible]
1964	378	1,388	18	5	8	11	6	2	448	630	.....	.....	2,[illegible]
1965	403	1,471	20	3	8	13	6	.....	456	388	.....	.....	2,7[illegible]
1966	370	1,734	38	14	8	38	4	4	491	491	.....	.....	3,192
1967	273	1,741	40	24	12	61	7	3	576	506	.....	.....	3,243
1968	248	1,842	41	21	10	72	2	2	601	487	.....	.....	3,[illegible]
1969	248	2,101	47	16	13	110	5	8	475	483	.....	.....	3,[illegible]
1970	276	2,182	45	16	12	85	(3)	7	430	546	.....	.....	3,[illegible]
1971[4]	168	2,047	56	15	5	140	1	9	520	517	.....	.....	3,479

[1] Includes fish oils which have been negligible in recent years. [2] Not reported separately beginning 1959. [3] Less than 500,000 pounds. [4] Preliminary.

Economic Research Service. Compiled from reports of the U.S. Department of Commerce. Totals computed from unrounded numbers. Data for 1944-48 in *Agricultural Statistics, 1967*, table 209.

Table 209.—Total fats and oils (including fat content of butter): Supply, disposition, and utilization, United States, 1949–71

Year	Supply: Production from domestic materials [1]	Supply: Imports and production from imported materials	Supply: Stocks, Jan. 1	Supply: Total	Disposition: Exports [1] [2]	Disposition: Domestic disappearance
	Million pounds	*Million pounds*	*Million pounds*	*Million pounds*	*Million pounds*	*Million pounds*
1949	11,828	1,104	[3] 1,696	14,628	2,248	[4] 10,15[illegible]
1950	12,056	1,320	[3] 2,178	15,554	2,045	[4] 11,323
1951	12,414	1,160	[3] 1,986	15,560	2,446	[4] 10,751
1952	12,341	1,001	[3] 2,[illegible]	15,7[illegible]	2,279	[4] 10,782
1953	12,806	1,001	[3] 2,664	16,471	2,655	10,8[illegible]
1954	13,269	994	[3] 3,012	17,275	3,943	11,0[illegible]
1955	14,364	1,056	[3] 2,264	17,684	4,102	[4] 11,[illegible]
1956	15,422	984	[3] 1,982	18,358	4,950	[4] 11,[illegible]
1957	15,275	1,008	[3] 1,734	18,017	4,670	[4] 11,6[illegible]
1958	15,049	1,017	[3] 1,662	17,798	3,593	[4] 12,[illegible]
1959	17,023	1,032	[3] 1,809	19,864	5,376	[4] 12,143
1960	17,352	1,075	[3] 2,325	20,752	5,965	12,649
1961	17,811	1,145	2,138	20,894	5,194	12,8[illegible]
1962	18,712	1,171	2,814	22,697	6,007	13,735
1963	19,338	1,104	3,193	23,635	6,551	13,584
1964	20,672	1,131	3,599	25,402	8,211	14,3[illegible]
1965	20,532	1,159	2,721	24,442	7,481	14,5[illegible]
1966	21,013	1,359	2,445	24,817	6,725	15,312
1967	21,064	1,316	2,794	25,114	7,248	15,145
1968	21,496	1,390	2,666	25,562	7,354	15,6[illegible]
1969	22,431	1,411	2,405	26,2[illegible]	7,613	16,2[illegible]
1970	23,024	1,4[illegible]	[illegible]	[illegible]	10,095	16,1[illegible]
1971	25,555	1,474	2,349	29,378	10,681	16,220

Year	Utilization (not adjusted for trade and change in stocks of shortening, margarine, soap, and secondaries): Food uses: Butter (fat content)	Food uses: Lard (direct)	Food uses: Shortening	Food uses: Margarine	Food uses: Other	Food uses: Total	Nonfood uses: Soap [7]	Nonfood uses: Drying-oil products	Nonfood uses: Other industrial	Nonfood uses: Total
	Million pounds	*Million pounds*	*Million pounds*	*Million pounds*	*Million pounds*	*Million pounds*	*Million pounds*	*Million pounds*	*Million pounds*	*Million pounds*
1949	1,273	1,792	1,444	791	1,1[illegible]	6,41[illegible]	1,817	974	[illegible]	[illegible]
1950	1,327	1,[illegible]	1,705	774	1,514	[illegible]	1,[illegible]	1,1[illegible]	1,[illegible]	4,2[illegible]
1951	1,213	1,[illegible]	1,405	651	1,187	6,[illegible]	1,571	1,151	1,[illegible]	4,6[illegible]
1952	1,102	1,[illegible]	1,613	1,[illegible]	1,051	6,958	1,416	1,032	1,[illegible]	3,714
1953	1,108	1,776	1,781	1,[illegible]	1,426	7,0[illegible]	1,349	1,[illegible]	1,240	3,[illegible]
1954	1,157	1,629	1,969	1,196	1,525	7,416	1,223	1,018	1,394	3,6[illegible]
1955	1,237	1,642	1,956	1,075	1,717	7,6[illegible]	1,158	1,121	1,673	3,[illegible]
1956	1,212	1,626	1,855	1,112	1,820	7,6[illegible]	1,054	1,107	1,915	4,[illegible]
1957	1,176	1,522	1,824	1,182	1,851	7,624	1,037	1,0[illegible]	2,017	4,0[illegible]
1958	1,162	1,642	2,010	1,269	1,844	7,947	948	934	2,262	4,14[illegible]
1959	1,145	1,528	2,234	1,291	2,028	8,2[illegible]	867	924	2,483	4,274
1960	1,112	1,361	2,352	1,367	2,094	8,2[illegible]	822	853	2,634	4,4[illegible]
1961	1,112	1,398	2,359	1,356	2,260	8,614	855	855	2,545	4,[illegible]
1962	1,131	1,320	2,696	1,394	2,534	9,075	796	876	3,147	4,[illegible]
1963	1,083	1,1[illegible]	2,611	1,451	2,470	8,909	797	860	3,188	4,[illegible]
1964	1,098	1,197	2,693	1,500	2,453	9,[illegible]	803	903	3,3[illegible]	5,072
1965	1,040	1,226	2,765	1,535	2,955	9,521	746	909	3,217	4,972
1966	911	1,072	3,192	1,710	2,917	9,802	731	928	3,749	5,[illegible]
1967	851	1,056	3,2[illegible]	1,7[illegible]	3,017	[illegible]	714	855	3,5[illegible]	[illegible]
1968	958	1,108	[illegible]	1,[illegible]	3,118	[illegible]	7[illegible]	869	3,[illegible]	5,4[illegible]
1969	908	1,012	3,505	1,744	3,[illegible]	10,[illegible]	759	775	3,6[illegible]	5,[illegible]
1970	868	937	3,598	1,764	3,945	11,161	759	827	4,[illegible]	5,[illegible]
1971	830	886	3,479	1,831	3,952	11,009	774	625	3,[illegible]	5,[illegible]

[1] Includes oil equivalent of cottonseed, soybeans, peanuts, and flaxseed exported for crushing abroad. [illegible] exports, voluntary or [illegible] year because of minor change in reporting procedures. [illegible] Government stockpiling. [illegible] tallow, and sperm oils held by the Government for exportation or storage in warehouses. [illegible] stockpile of coconut oil. [illegible] an estimate of oil equivalent of fats used in synthetic rubber, [illegible]

Economic Research Service. Computed from records of the Commerce, Interior, and Agriculture Departments. Totals computed from unrounded numbers. Data for 1944–48 in Agricultural Statistics, 1967, table 217.

Table 210.—Fats, oils, and oilseeds (fat or oil equivalent): World production and exports, 1968–71

Commodity	World production [1] 1968	1969	1970	1971 [2]	World exports [3] 1968	1969	1970 [2]
	1,000 metric tons	*1,000 metric tons*	*1,000 metric tons*	*1,000 metric tons*	*1,000 metric tons*	*1,000 metric tons*	*1,000 metric tons*
Edible vegetable oils: [4]							
Cottonseed	2,155	2,485	2,375	2,255	219	[illegible]	305
Peanut	3,[illegible]	3,025	3,[illegible]	3,305	1,139	865	810
Soybean	5,215	5,840	5,[illegible]	6,155	1,973	2,049	2,919
Sunflower	3,[illegible]	3,705	3,[illegible]	3,570	1,200	1,098	835
Rapeseed	1,905	1,475	1,[illegible]	2,415	350	386	481
Sesame	600	565	595	715	93	168	100
Safflower	285	175	[illegible]	225	31	24	23
Olive [5]	1,325	1,385	1,[illegible]	1,445	[6] 62	[6] 92	[6] 104
Corn	255	270	[illegible]	300	15	17	20
Total	18,710	19,925	19,545	20,305	5,163	4,927	5,587
Palm oils: [7]							
Coconut	2,150	2,040	2,110	2,600	1,247	1,072	1,093
Palm kernel	365	385	410	465	300	317	331
[illegible]	1,405	1,570	1,[illegible]	2,015	668	[illegible]	739
Babassu kernel [8]	65	101	102	107	9	15	17
Total	3,985	4,096	4,412	4,987	2,224	2,142	2,209
Industrial oils:							
Linseed [9]	785	920	1,140	1,243	354	428	420
Castor [9]	365	345	350	325	228	250	298
[illegible]	29	2	15	0	11	7	10
Tung	125	130	117	120	42	48	35
Olive residue [10]	146	148	115	141	(11)	(11)	(11)
Total	1,450	1,565	1,738	1,639	635	730	701
Animal fats:							
Butter (fat content)	4,050	4,000	3,850	3,900	481	491	503
Lard [12]	4,[illegible]	3,900	4,305	4,200	421	442	443
Tallow and greases [13]	4,050	4,255	4,425	4,620	1,446	1,417	1,557
Total	12,365	12,215	12,580	12,720	2,348	2,350	2,504
Marine oils:							
Whale	92	76	69	60	38	26	22
Sperm whale	122	150	140	125	90	95	64
Fish (including liver)	1,110	914	1,052	1,086	[6] 723	[6] 627	[6] 558
Total	1,324	1,120	1,261	1,292	850	748	654
Total all commodities	37,834	37,921	39,736	41,373	11,230	10,896	11,716

[1] Years indicated are those in which the predominant share of the given oil or fat was produced from its related raw material.
[2] Preliminary.
[3] Exports from producing countries.
[4] Production series revised for all commodities except olive and corn oils.
[5] Excludes olive residue oil.
[6] Net exports.
[7] Production estimated on the basis of exports and information available on consumption in the various producing areas.
[8] Mill production only.
[9] Production series revised.
[10] Includes quantities of refined oil for edible purposes.
[11] Not separately classified.
[12] Production relates to rendered lard only in most countries.
[13] Export series revised; figures include edible and inedible tallow and greases but exclude animal oils.

Foreign Agricultural Service. Prepared or estimated on the basis of official statistics of foreign governments, other foreign source materials, reports of U.S. Agricultural Attaches and Foreign Service Officers, results of office research, and related information.

Table 211.—Fats, oils, oilseeds, and oilseed cake and meal: Exports of selected items, United States, 1954–70

Year beginning July	Lard	Inedible animal tallow, greases, and oils [1]	Tallow, edible	Oilseeds and nuts: Cottonseed	Oilseeds and nuts: Flaxseed	Oilseeds and nuts: Peanuts unshelled	Oilseeds and nuts: Peanuts shelled	Oilseeds and nuts: Soybeans
	1,000 pounds	*1,000 pounds*	*1,000 pounds*	*1,000 pounds*	*1,000 pounds*	*1,000 pounds*	*1,000 pounds*	*1,000 pounds*
1954	516,123	1,240,991	9,649	42,333	457,556	533	7,298	3,065,220
1955	637,462	1,457,638	19,973	31,617	564,864	192	1,378	[illegible]
1956	561,335	1,469,234	9,255	22,198	142,702	14,374	55,873	4,601,165
1957	409,006	1,160,667	5,898	12,246	505,957	3,504	28,191	5,276,168
1958	465,876	1,204,334	5,866	7,580	335,302	2,680	37,732	6,159,730
1959	673,600	1,712,270	18,338	17,004	466,236	4,000	51,761	7,913,440
1960	490,059	1,650,862	5,946	11,118	359,917	375	54,340	8,590,249
1961	432,879	1,820,539	5,624	11,868	54,069	380	25,944	8,648,355
1962	444,276	1,690,708	4,716	22,414	242,756	1,860	25,958	10,260,056
1963	668,412	2,179,239	3,686	14,928	200,237	1,013	64,788	11,254,106
1964	442,514	2,283,010	4,952	13,772	365,358	8,773	117,334	12,519,947
1965	182,505	1,985,906	3,362	18,040	295,104	5,864	177,820	15,395,030[6]
1966	108,940	2,149,506	6,122	10,424	418,026	652	137,699	15,107,023
1967	[illegible]	2,183,123	4,757	6,525	242,456	390	144,935	[illegible]
1968	209,330	2,113,337	9,705	7,562	545,196	721	79,694	17,442,706
1969 [2]	302,480	2,024,675	6,258	37,521	320,919	1,515	99,901	24,365,903
1970 [2]	386,590	2,440,865	10,931	87,947	186,548	6,004	196,822	25,454,204

Year beginning July	Vegetable oils: Cocoa butter	Vegetable oils: Coconut oil	Vegetable oils: Cottonseed oil [3]	Vegetable oils: Linseed oil	Vegetable oils: Margarine	Vegetable oils: Peanut oil	Vegetable oils: Soybean oil [4]	Oilseed cake and meal: Soybean	Oilseed cake and meal: Other
	1,000 pounds	*1,000 pounds*	*1,000 pounds*	*1,000 pounds*	*1,000 pounds*	*1,000 pounds*	*1,000 pounds*	*Tons*	*Tons*
1954	8,067	10,337	[3]654,335	317,579	[illegible]	889	35,158	[illegible]	297,201
1955	1,853	8,763	[3]665,025	[illegible]	4,234	1,077	371,340	401,349	372,293
1956	1,829	8,707	[3][illegible]	[illegible]	[illegible]	25,493	928,332	447,124	138,961
1957	649	9,829	[3]298,548	67,152	4,523	4,515	[4]627,438	317,424	27,325
1958	310	7,872	284,754	8,175	4,053	6,731	795,525	456,464	22,620
1959	141	8,655	560,870	34,949	4,326	13,051	1,029,709	635,904	228,566
1960	356	4,467	404,402	23,640	3,595	10,332	856,879	595,063	104,596
1961	111	2,114	426,492	1,664	3,024	[5]11,016	[illegible]	[illegible]	28,163
1962	230	956	406,823	8,770	2,994	2,014	1,292,642	1,391,479	132,566
1963	112	5,576	475,351	14,392	2,995	41,159	1,032,454	1,320,040	[illegible]
1964	68	2,332	684,225	19,949	2,967	94,529	1,368,990	2,124,110	234,216
1965	300	4,502	389,210	63,702	3,509	21,732	1,009,833	2,524,447	252,741
1966	364	4,530	76,212	120,858	3,973	3,469	1,051,727	[illegible]	149,296
1967	249	5,212	50,652	52,171	5,042	6,172	[illegible]	2,556,730	154,459
1968	526	4,770	113,701	76,966	3,754	11,226	802,027	2,990,221	169,797
1969 [2]	1,544	6,153	443,276	43,006	4,040	47,643	1,164,702	3,753,614	155,451
1970 [2]	2,313	10,850	304,358	52,496	2,967	30,527	1,772,451	4,223,921	209,591

[1] Includes inedible tallow, inedible animal oils not elsewhere classified, and inedible animal greases and fats, not elsewhere classified, through 1964. Beginning 1965, data include tallow and grease (except wool grease) and choice white grease; lard and tallow oil; lard, oleo, and tallow stearine; unrendered animal fats, and animal fats and oils, not elsewhere classified.

[2] Preliminary.

[3] Includes the following estimated quantities exported under Title III, P.L. 480 (Foreign Donations) (thousand pounds): 12,232 in 1960, 69,932 in 1961, 30,207 in 1962, 1,426 in 1963, and 107,705 in 1964. Beginning January 1965, donations included as reported by the Census Bureau.

[4] Includes the following estimated quantities exported under Title III, P.L. 480 (Foreign Donations) (thousand pounds): 38,128 in 1960, 152,781 in 1961, 92,456 in 1962, 18,004 in 1963, and 74,379 in 1964. Beginning January 1965, donations included as reported by the Census Bureau.

[5] Includes food exported for relief or charity.

[6] Includes an estimated 10,192 thousand pounds exported under Title III, P.L. 480 (Foreign Donations).

Foreign Agricultural Service. Compiled from reports of the Commerce and Agriculture Departments. Data for 1949-53 in Agricultural Statistics, 1967, table 214.

Table 215.—Fats and oils: Use in products for civilian consumption, United States, 1949–71

Year	Food products: Butter (actual weight): Total	Butter: Per capita	Lard excluding use in margarine, shortening, and nonfood products: Total	Lard: Per capita	Margarine (actual weight): Total	Margarine: Per capita	Shortening: Total	Shortening: Per capita	Edible oils [1]: Total	Edible oils: Per capita	All food products [2]: Total	All food products: Per capita
	Million pounds	*Pounds*	*Million pounds*	*Pounds*	*Million pounds*	*Pounds*	*Million pounds*	*Pounds*	*Million pounds*	*Pounds*	*Million pounds*	*Pounds*
1949	1,549	10.5	1,744	11.8	851	5.8	1,435	9.7	1,162	7.9	6,257	42.6
1950	1,614	10.7	1,891	12.6	918	6.1	1,656	11.0	1,297	8.6	6,890	45.9
1951	1,445	9.6	1,855	12.3	996	6.6	1,365	9.0	1,153	7.7	6,366	42.1
1952	1,316	8.6	1,817	11.8	1,219	7.9	1,562	10.2	1,339	8.7	6,765	44.1
1953	1,329	8.5	1,772	11.4	1,256	8.1	1,597	10.2	1,415	9.1	6,876	44.1
1954	1,410	8.9	1,627	10.2	1,346	8.5	1,870	11.8	1,514	9.5	7,228	45.5
1955	1,466	9.0	1,639	10.1	1,323	8.2	1,863	11.5	1,704	10.5	7,447	45.9
1956	1,439	8.7	1,623	9.8	1,354	8.2	1,797	10.9	1,800	10.9	7,475	45.2
1957	1,406	8.4	1,589	9.4	1,446	8.6	1,756	10.4	1,824	10.8	7,469	44.4
1958	1,418	8.3	1,640	9.6	1,549	9.0	1,935	11.3	1,804	10.5	7,770	45.3
1959	1,371	7.9	1,536	8.8	1,604	9.2	2,186	12.6	1,945	11.2	8,068	45.2
1960	1,332	7.5	1,359	7.7	1,675	9.4	[illegible]	12.6	2,034	11.5	8,055	45.2
1961	1,325	7.4	1,343	7.7	1,705	9.4	2,311	12.8	2,022	11.2	8,173	45.1
1962	1,342	7.3	1,314	7.2	1,706	9.3	2,469	13.4	2,155	11.7	[illegible]	45.7
1963	1,281	6.9	1,190	6.4	1,785	9.6	2,525	13.5	2,450	13.1	[illegible]	46.3
1964	1,297	6.8	1,193	6.3	1,835	9.7	[illegible]	13.7	2,724	14.4	8,904	47.5
1965	1,232	6.4	1,235	[illegible]	[illegible]	9.9	2,645	14.0	2,711	14.1	9,147	47.7
1966	1,099	5.7	1,071	5.5	[illegible]	10.5	3,079	15.9	2,973	15.3	9,559	49.8
1967	1,076	5.5	1,055	5.4	2,045	10.5	3,108	15.9	2,956	15.1	9,672	49.2
1968	1,117	5.7	1,106	5.6	[illegible]	10.5	3,211	[illegible]	[illegible]	16.0	[illegible]	51.0
1969	1,061	5.4	1,011	5.1	2,154	10.8	3,398	17.0	3,736	16.7	10,303	51.9
1970	1,061	5.2	939	4.7	[illegible]	11.0	3,496	[illegible]	[illegible]	18.2	10,741	53.3
1971 [3]	1,034	5.1	887	4.3	2,264	11.1	3,429	16.8	3,695	18.0	10,653	52.2

Year	Industrial products: Soap [4]: Total	Soap: Per capita	Drying-oil products [5]: Total	Drying-oil products: Per capita	Other industrial products [6]: Total	Other industrial products: Per capita	All industrial products: Total	All industrial products: Per capita	All products: Weight: Including actual weight of butter and margarine	All products: Weight: Including only fat content of butter and margarine	All products: Per capita [2]
	Million pounds	*Pounds*	*Million pounds*	*Pounds*	*Million pounds*	*Pounds*	*Million pounds*	*Pounds*	*Million pounds*	*Million pounds*	*Pounds*
1949	1,738	11.9	[illegible]	6.5	856	5.8	3,576	24.2	10,318	9,853	66.8
1950	[illegible]	12.0	1,182	7.9	1,153	7.7	4,140	27.6	11,416	11,030	73.4
1951	1,506	10.0	1,129	7.5	1,217	8.1	3,850	25.5	10,679	10,216	67.6
1952	1,352	8.8	[illegible]	6.6	1,271	8.0	[illegible]	23.4	10,646	10,358	67.5
1953	1,291	8.3	1,064	6.8	1,274	8.2	[illegible]	23.3	11,001	10,507	67.4
1954	1,177	7.4	1,001	6.3	1,349	8.5	3,527	22.2	11,295	10,765	67.7
1955	1,115	6.9	1,104	6.8	1,621	10.0	3,840	23.7	11,829	11,287	69.5
1956	1,058	6.3	[illegible]	6.6	[illegible]	11.1	[illegible]	24.0	11,976	11,437	69.2
1957	994	5.9	1,015	6.0	[illegible]	11.6	[illegible]	23.5	11,940	11,428	67.9
1958	914	5.3	920	5.4	2,135	12.6	[illegible]	22.3	12,335	[illegible]	68.6
1959	862	4.9	[illegible]	5.2	[illegible]	14.1	4,245	24.3	12,869	[illegible]	70.5
1960	860	4.8	821	4.5	2,590	14.5	4,271	24.0	12,911	[illegible]	69.2
1961	830	4.6	846	4.7	[illegible]	14.1	[illegible]	23.3	12,944	[illegible]	68.4
1962	774	4.2	867	4.7	3,073	16.7	4,714	25.6	13,706	13,117	71.4
1963	775	4.2	[illegible]	4.6	3,154	16.9	[illegible]	25.6	14,030	13,421	71.9
1964	773	4.1	[illegible]	4.7	3,313	17.5	4,972	25.3	14,574	[illegible]	73.8
1965	708	3.7	[illegible]	4.6	3,275	16.8	4,816	25.1	14,201	[illegible]	72.8
1966	719	3.7	[illegible]	4.7	3,703	19.1	5,329	27.5	15,233	[illegible]	77.3
1967	706	3.6	844	4.3	[illegible]	18.8	[illegible]	[illegible]	15,475	[illegible]	78.0
1968	689	3.5	[illegible]	4.3	[illegible]	18.8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1969	[illegible]	3.4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1970	679	3.4	[illegible]	3.1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1971 [3]	677	3.3	[illegible]	3.0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

LARD

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Nutritional Evaluation of Some Heated Oils[1,2]

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THE TOXIC NATURE of heat-abused oils has been the subject of much discussion in the literature. Fractionation of these oils has yielded various polymerized products which, when fed to rats, have resulted in interferences with good nutrition and the appearance of pathological symptoms.

Polymerized oils have been prepared in many ways, by different methods of treating the oils and by using various types of oils as starting materials. Many conflicting reports of the impaired nutrition resulting from the ingestion of these polymers are available. Crampton and co-workers (1) heated soybean and sunflower seed oil anaerobically for 24-26 hrs. and reported the production of a toxic substance resulting from the polyunsaturated nature of the fatty acids of these oils. Johnson and co-workers (2), in experiments in which rats were fed diets containing heated corn oil, heated margarine and heated butter fat, confirmed the observation that the toxic effect of the heated oils resulted from their polyunsaturated fatty acid content since in animals fed the heated corn oil the growth rate was considerably lower than in the control animals receiving fresh oil; in animals fed heated margarine only moderately reduced growth was observed as compared with the control animals whereas the ingestion of heated butter fat had no deleterious effect on growth. In all of these groups however no permanent metabolic damage was produced. Experiments in which heat-polymerized menhaden oil and linseed oil were fed to rats also resulted in the observation that the toxicity was associated with polymerized polyunsaturated fatty acids contained in these oils (3).

These interferences with nutrition can be remedied. In experiments reported by Kaunitz (4) the toxicity induced in rats by feeding a heat-treated oxidized cottonseed oil was partially nullified by the addition of fresh oil to the diet. Other investigators reported that the toxicity of thermally polymerized fats could be partially overcome by supplementation with pyridoxine (5).

Recently studies have become available which indicate that the laboratory heat-treatment of oils, on which the above reports are based, are unrealistically drastic. Melnick (6) has reviewed critically these earlier reports and has concluded that the findings are irrelevant to practical operations. Thus, in a survey by Melnick and associates (6-8) of operations by 86 different potato chip manufacturers, it was shown that the frying oils and fats used in these operations had only a 1% decrease in iodine value, which was of no nutritional significance. Results from our laboratory have shown that heated margarine fat caused no decrease in nutritional value in tests conducted on rats (9). Rice and co-workers (10) reported studies of 34 samples of used fats obtained from restaurants, bakeries, and potato-chip producers and showed that even the most severe usage caused only slight decreases in caloric availability and slight increases in liver size in tests with rats.

TABLE I
Description of Oils

Oil or fat	Conditions of heating	I.V.	Decrease in I.V. resulting from heating %	Viscosity (centistokes at 27°C.)
Soybean oil	.....	128		52
Soybean oil H.P.	610°F. 70 min.	120	6	92
Soybean oil H.P.	610°F. 100 min.	116	10	114
Cottonseed oil	.....	112		55
Cottonseed oil H	610°F. 70 min.	106	5	83
Lard	400°F. 60 min.	61		67
Lard H	610°F. 70 min.	59	3	78

The investigation reported here was undertaken to evaluate the nutritional quality (and safety) of several heated fats, containing different concentrations of polyunsaturated fatty acids, using unheated samples as controls. The investigation was limited to a study of thermal nonoxidative polymers prepared under rigid laboratory conditions. It was desired to relate changes in physical constants, such as viscosity and iodine value, with several nutritional indices. It has been our experience that diets which seem to be adequate for growth and maintenance may be quite unsatisfactory for reproduction where different types of stresses are brought to bear on the animal. The survival of groups of these animals was followed over their life span, and when a 75% mortality was reached in each group, the animals were sacrificed and analyses of tissues for cholesterol were conducted.

Experimental

Soybean oil, cottonseed oil, and lard[3] were heated as described below. The changes in various physical constants are shown in Table I. Heat was applied to these samples *in vacuo* to reduce peroxide formation

[3] The soybean and cottonseed oils were commercially refined and were purchased through regular trade channels. The lard was purchased from Armour and Company and was refined and bleached in the pilot plant of the Glenview Laboratories of the National Dairy Products Corporation. All heated oils were subject to deaeration since this is part of the deodorization process.

TABLE II
Basal Diet

Constituent	% of Diet
Casein	24
Cellulose	4
Salt mixture[a]	4
Fat	15
Sucrose	52.5
Choline	0.24
Dry vitamin mix[b]	0.19
Mixed tocopherols (34%)	0.03
Vitamin A	10,000 units/kg diet
Vitamin D	2,000 units/kg diet
Vitamin B_{12}	60 μg/kg diet

[a] Wesson modification of Osborne-Mendel salt mixture.
[b] Dry Vitamin Mix: Thiamin HCl 4.59, Riboflavin 1.72, Niacin [illegible], Folic acid 0.638, Pyridoxine 1.72, Ca pantothenate 3.82, Menadione 0.319, Biotin 0.159, Vitamin C 12.7, P-aminobenzoic acid 38.6, Inositol 31.9.

[1] Presented at the fall meeting, American Oil Chemists' Society, Chicago, Ill., October 21, 1958.
[2] Contribution No. 367.

TABLE III

Food Consumption, Food Efficiency, and Digestibility of Various Heated and Unheated Fats

Diet	S.O.	$S.O._{L.P.}$	$S.O._{H.P.}$	Lard	$Lard_H$	CSO	CSO_H
Food consumption (g.)							
Weeks 1– 2	63	69	63	72	65	66	67
3– 4	93	95	88	91	97	95	97
5– 6	105	103	107	113	100	109	107
7– 8	103	104	103	119	114	115	107
9–10	97	105	103	108	103	96	96
Food efficiency							
Gain in wt./g. food eaten							
Week 2	0.63	0.47	0.49	0.43	0.53	0.50	0.58
4	0.36	0.31	0.30	0.33	0.33	0.36	0.37
6	0.27	0.24	0.28	0.16	0.17	0.23	0.23
Digestibility (%)	98	93	91	93	94	96	93

Values are the averages of results obtained from 15 male rats per group.

The control soybean oil (S.O.) was deaerated and packed directly into gallon cans which had been flushed with nitrogen. The low-polymer soybean oil ($S.O._{L.P.}$) was heated at 610°F. for 70 min. at a pressure of 2 millimeters. A small amount of steam was introduced for agitation. The oil was then cooled and packed directly into nitrogen-flushed cans. The high-polymer soybean oil ($S.O._{H.P.}$) was treated similarly except for the longer heating period (100 min.).

The control cottonseed oil (CSO) was treated similarly to the control soybean oil. The treatment and handling of the heated cottonseed oil (CSO_H) was identical to that given the low-polymer soybean oil.

The control lard was 100% leaf lard which, after refining and bleaching, was subjected to a light deodorization at 400°F. for 60 min. at 2 mm. of pressure. The heated lard product was obtained from the control lard by processing under conditions identical with those used for the low-level polymer soybean oil.

All fats were included in the diet at the level of 15% (Table II). The diets were fed to groups of 30 male and 30 female rats from weaning.

TABLE IV

Reproductive Performance of Female Rats Fed Heated and Unheated Cottonseed Oil and Heated and Unheated Lard

Category	CSO	CSO_H	Lard	$Lard_H$
Females bred	14	14	14	14
Litters cast (%)	93	100	72	72
Litters (at birth)				
Total No. rats	124	153	80	93
No./litter	9.5	10.9	8.0	9.3
Litters (at 3 days)				
Total No. rats	91	101	59	52
Av. wt./rat	6.6	7.0	6.7	6.4
Litters (at 21 days)*				
Total No. rats	56	63	53	38
Av. wt./rat	34.5	31.9	33.8	31.4
Mortality				
0– 3 days	27	27	36	44
4–21 days	20	15	2	7

* Litters were cut to seven at three days.

Results and Discussion

Food consumption was measured during the first 10 weeks, and food efficiency was calculated for six weeks. The results are shown in Table III. No significant differences were observed in the animals fed the heated fats as compared with the control animals and the unheated fats. Digestibility measurements however indicated that small decreases in digestibility were obtained with the heated soybean and cottonseed fats (Table III).

Growth in all groups in both male and female rats was little affected by the presence of the heated fats in the diet (Figure 1). The gain in weight is charted over a four-month period during the active growth of the animals. Although only a slight depression in growth in the animals fed the diet containing the highly polymerized soybean oil was observable at the end of four months, the interference with growth in this group became more pronounced with time.

TABLE V

Reproductive Performance of Female Rats Fed Unheated Soybean Oil and Heated Soybean Oil with and without Supplements of Linoleate or α Tocopherol

Category	S.O.	$S.O._{L.P.}$	$S.O._{H.P.}$	$S.O._{H.P.}$ + Linoleate	$S.O._{H.P.}$ + α-tocopherol
Females bred	15	15	15	15	15
Litters cast (%)	100	93	80	87	100
Litters (at birth)					
Total No. rats	159	141	110	141	176
No./litter	10.6	10.1	9.2	10.1	11.7
Litters (at 3 days)					
Total No. rats	141	121	72	120	157
Av. wt./rat	7.3	6.9	6.8	6.7	7.3
Litters (at 21 days)*					
Total No. rats	96	88	59	69	95
Av. wt./rat	33.4	32.9	34.6	38.4	36.5
% Mortality 0– 3 days	11	16	34	8	11
4–21 days	2	15	0	18	10

* Litters were cut to seven at three days.

When male rats on the experimental diets were mated with female rats of our stock colony, there was no indication of any interference with gonadal development or maturation of sperm. Reproduction and lactation were normal. However when female rats on experimental diets were mated with male rats of our stock colony, although no significant differences in reproductive performance were obtained with animals fed unheated cottonseed oil as compared with the heated oil or unheated lard as compared with heated lard (Table IV), an interference with reproduction was observed in the animals fed the highly polymerized soybean oil (Table V). This was most evident in the number of young born and in the survival of the young at three days. In an attempt to determine the nature of this interference with pregnancy and lactation, female rats on the high-polymer soybean oil diet were supplemented from weaning with either 100 mg.

TABLE VI

Time in Weeks at Which 25, 50, and 75% Mortality of Male and Female Rats, Fed Heated and Unheated Fats, Was Reached

Diet		LD_{25} Wks.	LD_{50} Wks.	LD_{75} Wks.	Incidence of cysts and tumors
S.O.	M	75	97	116	0
	F	87	99	116	4
$S.O._{L.P.}$	M	75	108	115	0
	F	87	101	115	6
$S.O._{H.P.}$	M	82	100	117	1
	F	60	97	116	4
CSO	M	57	91	112	3
	F	77	107	112	9
CSO_H	M	82	110	116	2
	F	87	97	115	3
Lard	M	65	97	116	0
	F	60	88	111	7
$Lard_H$	M	72	108	115	1
	F	82	109	114	6

TABLE VII

Cholesterol Levels in Plasma and Liver in Aged Male Rats Fed Various Fat Diets

Diet	Age at sacrifice[a]	Wt. at sacrifice	Plasma Cholesterol Free[b]	Plasma Cholesterol Total[b]	Plasma Cholesterol % F.	Wt. % of body wt.	Liver Cholesterol Free[b]	Liver Cholesterol Total[b]	Liver Lipid Total[b]
	weeks	*g.*	*mg. %*	*mg. %*			*mg./g.*	*mg./g.*	*mg./g.*
S.O.	116(5)	433	24 ± 1	81 ± 5	32	2.3	2.3 ± 0.1	3.6 ± 0.3	52.5 ± 6.1
S.O.H.P.	115(6)	470	28 ± 5	67 ± 8	42	2.4	2.2 ± 0.1	3.2 ± 0.4	56.9 ± 4.3
S.O.H.P.	117(6)	389	26 ± 2	78 ± 6	33	3.1	2.5 ± 0.1	3.4 ± 0.4	46.4 ± 6.1
Lard	116(6)	451	25 ± 3	80 ± 3	31	2.5	2.0 ± 0.1	3.0 ± 0.2	50.9 ± 2.8
Lard H	111(6)	466	31 ± 3	88 ± 5	35	2.4	2.0 ± 0.1	2.9 ± 0.1	40.3 ± 1.7
CSO	116(3)	380	41 ± 9	108 ± 7	33	2.5	2.5 ± 0.1	3.0 ± 0.1	48.8 ± 10.6
CSO H	116(6)	415	33 ± 2	84 ± 10	40	2.6	2.2 ± 0.1	2.8 ± 0.2	41.2 ± 2.2

[a] Numbers in parentheses are numbers of animals in each group.
[b] Including standard error of the mean.

of the essential fatty acid ester, linoleate, or with twice the amount of α tocopherol normally included in the diet. These animals were then bred with males of our stock colony as before. Although a slight improvement in reproductive performance was noted as a result of linoleate supplementation, the addition of increased Vitamin E yielded results comparable to those obtained with the unheated oil, indicating that the ingestion of this highly polymerized soybean oil increased the requirement for this vitamin.

The survivals of rats fed the heated oils and the fresh oils were practically the same in all cases (Table VI). A 75% mortality was obtained between 111 and 117 weeks. There was no evidence of increased tumor incidence in the groups ingesting the heated oils.

The results of the cholesterol determinations in plasma and liver of the surviving male and female animals are shown in Tables VII and VIII. Nishida *et al.* (11) have reported a depression in serum cholesterol levels as a result of the substitution of heated oil for fresh oil in the diet. Similar results were evident only in female rats fed the diet containing heated lard and in male rats ingesting the heated cottonseed oil diet. In all the other groups plasma cholesterol levels were unaffected by the substitution of heated for unheated fats.

The concentration of cholesterol in liver of these animals was very similar in all cases. Total lipid values were similarly unchanged by the substitution of heated fats for unheated fats in the diet. However the weight of the liver of male rats fed the highly polymerized soybean oil expressed as percentage body weight, was higher than in the other groups; this increase was not reflected by elevations in cholesterol and total lipid but resulted from the lower body weight observed in these animals.

Summary

Soybean oil, cottonseed oil, and lard were heated *in vacuo* for either 70 and/or 100 min. at 610°F. and were fed to male and female rats at a level of 15% in the diet over a complete life span. Nutritional indices such as growth, including food efficiency and digestibility of the fats, reproduction and lactation, longevity, and tissue cholesterol and total lipid were measured. No evidence of impaired nutrition or harmfulness was observed in any group except in those fed the more highly polymerized soybean oil, where a decrease in iodine value of 10% and a 100% increase in viscosity was associated with a slight depression in growth and an interference with reproductive performance in the female animal. This interference with the reproductive process was alleviated by supplementation with α-tocopherol. There was no evidence of increased tumor incidence in the groups ingesting the heated oils. Digestibility of the heated soybean and cottonseed oils was only slightly reduced.

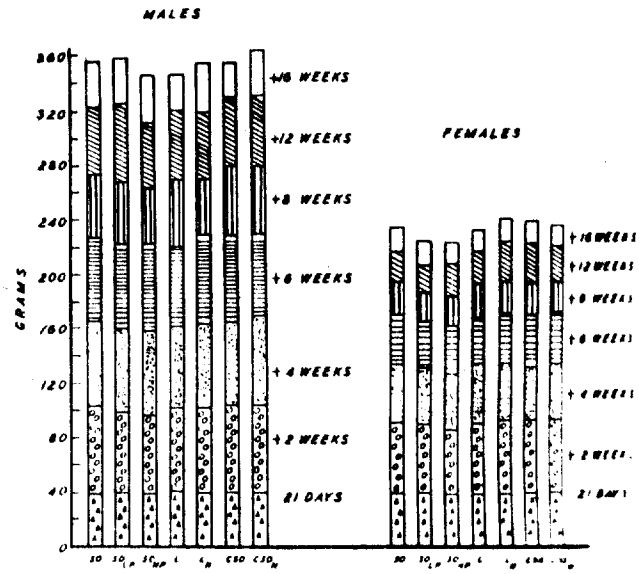

FIG. 1. Growth over a four-month period of male and female rats fed from weaning various unheated and heated fats.

TABLE VIII

Cholesterol Levels in Plasma and Liver in Aged Female Rats Fed Various Fat Diets

Diet	Age at sacrifice[a]	Wt. at sacrifice	Plasma Cholesterol Free[b]	Plasma Cholesterol Total[b]	Plasma Cholesterol % F.	Wt. % of body wt.	Liver Cholesterol Free[b]	Liver Cholesterol Total[b]	Liver Lipid Total[b]
	weeks	*g.*	*mg. %*	*mg. %*			*mg./g.*	*mg./g.*	*mg./g.*
S.O.	116(5)	288	22 ± 1	67 ± 4	33	3.0	2.2 ± 0.1	2.8 ± 0.2	45.0 ± 5.7
S.O.H.P.	115(6)	348	23 ± 2	86 ± 5	26	3.2	2.1 ± 0.1	2.6 ± 0.1	42.2 ± 3.1
S.O.H.P.	116(6)	348	34 ± 4	85 ± 11	41	2.8	2.4 ± 0.1	2.6 ± 0.1	40.1 ± 2.4
Lard	115(6)	362	31 ± 3	104 ± 5	30	2.7	2.1 ± 0.1	2.5 ± 0.2	42.8 ± 4.2
Lard H	116(4)	370	21 ± 1	52 ± 2	40	2.8	2.2 ± 0.1	2.9 ± 0.2	46.2 ± 0.7
CSO	112(6)	388	30 ± 2	80 ± 6	38	3.0	1.9 ± 0.1	2.4 ± 0.1	34.5 ± 1.6
CSO H	115(4)	418	29 ± 5	77 ± 12	39	2.4	2.2 ± 0.2	3.1 ± 0.2	53.6 ± 2.8

[a] Numbers in parentheses are numbers of animals in each group.
[b] Including standard error of the mean.

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THE PRODUCTION OF DIBENZANTHRACENE TUMORS IN PURE STRAIN MICE

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Burrows, Hieger, and Kennaway (1) have shown that the compound 1:2:5:6-dibenzanthracene, when injected subcutaneously in lard solution, is capable of producing sarcomas in mice. In their experiments the compound induced tumors in 31 out of 93 mice. Seven primary growths were used for serial transmission experiments, of which two were carried at least as far as the twelfth and sixteenth generation.

Because of the inconsistent results obtained in their transmission experiments and the fact that no mention was made of any particular strain of mice, it is assumed that Burrows, Hieger, and Kennaway did not use pure strain animals. Therefore, it was considered of interest to ascertain the results attending the injection of 1:2:5:6-dibenzanthracene into pure strain mice. The purpose of such an experiment would be twofold: First, to determine whether the compound is capable of inducing tumors in pure strain animals which exhibit a low incidence of spontaneous tumors, as well as in other strains showing a high incidence of spontaneous tumors; second, to determine whether these induced tumors in pure stocks would follow the rule of the genetic theory of transplantation, namely, that a spontaneous tumor arising within an individual of a strain can be transplanted to members of the same strain, but not to members of another strain. This report deals briefly with the results of a single experiment conducted along these lines.

EXPERIMENTAL ANIMALS

All pure strain mice were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. The mice used in the experiment are described below.

Strain A.—Inbred since 1918. Albino mice with a high incidence of spontaneous tumors in breeding females.

Strain M "Leaden."—Inbred since 1921. Color the same as strain D to be described below. These mice show a low incidence of spontaneous tumors.

Strain C_3H.—Inbred since 1921. Color of wild house mice. The breeding females have a high incidence of mammary carcinomas.

Strain CBA.—Inbred since 1921. Color of wild house mice. No tumor has been observed in the mice of this strain for the past 10 generations.

Strain D. Inbred since 1909. Dilute brown color. Breeding females exhibit an extraordinarily high incidence of spontaneous tumors.

Stock mice.—Mice purchased from a local dealer. Albino mice were used to compare the reaction of "market mice" to pure strain mice when subjected to injections of dibenzanthracene-lard solution.

Only adult mice weighing at least 20 g were used. All female mice were virgins.

TECHNIQUE

A solution of 1:2:5:6-dibenzanthracene in lard was prepared as follows: The lard was filtered at 38° C., and dibenzanthracene was then added in the proportion of 4 mg to each cubic centimeter of lard. The lard was heated to 140° C., at which temperature the compound was completely dissolved. The control lard was also heated to 140° C. Both the dibenzanthracene-lard solution and the control lard were cooled to room temperature and then kept at +4° C. until used. Before using, both were heated to 40° C.

The injections were made by means of an 18-gage needle and a 1-cc syringe. All injections were made subcutaneously in the right axillary region.

EXPERIMENTAL OBSERVATIONS

The experimental animals consisted of 558 mice, distributed among the various strains as follows:

Strain	Number of experimental animals	Number of controls
Strain A	125	63
Strain M	102	50
Strain C_3H	19	10
Strain CBA	58	31
Strain D	23	13
Stock	41	22

The time of injections and amounts given were as follows:

	Cubic centimeter
Aug. 3, 1933	0.25
Aug. 18, 1933	0.25
Nov. 1, 1933	0.50

The first two injections produced subcutaneous lumps which persisted without showing any evidence of being absorbed. Therefore, on October 24, 1933, these masses were broken by pressure. What bearing this procedure had on the final outcome of the experiment is unknown.

The first tumor was noted on November 16, 1933, only 15 days after the last injection. Hence the necessity for the final injection was not established.

Following the appearance of the first tumor, the mice were examined each week, with the exception of the 17th, 21st, and 24th weeks following the first injection. As a routine procedure, any mouse dying was

autopsied and examined macroscopically for the presence of tumor. Pieces from every tumor were fixed in Tellyesniczky's fluid.

The experiment was discontinued on February 8, 1934, just 27 weeks after the initial injection. The results of the experiment are shown in table 1. The lard-control mice are omitted from the table, since none developed tumors during the entire period of observation.

TABLE 1.—*Results of injection of dibenzanthracene in lard*

Strain	Sex	Number of mice injected	Died from other causes	Number of mice developing tumor	Percent	Living on Feb. 8, 1934
A	Male	60	21	27	45	12
A	Female	65	16	31	48	18
M	Male	30	4	20	67	6
M	Female	72	18	18	25	36
C3H	Male	9	4	5	55	0
C3H	Female	10	4	6	60	0
CBA	Male	22	12	8	36	2
CBA	Female	36	8	23	64	5
Stock	Male	12	10	2	16	0
Stock	Female	29	11	7	24	11
D	Male	23	3	6	26	14
Total		368	111	153	41	104

It is seen that the dibenzanthracene-lard solution induced tumors in all five pure strain stocks as well as in the "market mice".

The time of appearance of tumors is shown in table 2. It is seen that the greatest number were observed from the nineteenth to the twenty-sixth week.

TABLE 2.—*Time in weeks of the appearance of dibenzanthracene-lard tumors in mice*

Time in weeks		15	16	17	18	19	20	21	22	23	24	25	26	27	Total number of tumors
Strain	Sex	Numbers of tumors observed													
A	Female	2	1	...	3	3	2	...	5	5	...	6	3	1	31
A	Male	2	1	...	...	1	3	...	5	3	...	7	5	...	27
C3H	Female	1	1	...	...	1	1	...	1	1	...	...	...	...	6
C3H	Male	1	1	...	...	2	1	...	...	...	...	...	...	...	5
CBA	Female	1	...	...	2	1	3	...	5	4	...	4	2	1	23
CBA	Male	...	...	...	...	...	...	...	1	2	...	4	1	...	8
M	Female	...	...	...	...	...	1	...	4	1	...	6	5	1	18
M	Male	...	...	...	1	2	2	...	6	5	...	1	2	1	20
Stock	Female	1	...	...	1	2	...	...	2	...	...	...	1	...	7
Stock	Male	1	...	...	...	...	...	...	1	...	...	...	...	...	2
D	Male	...	...	...	1	...	...	...	3	1	...	1	...	...	6
Total		9	4	...	8	12	13	...	33	22	...	29	19	4	153

LUNG TUMORS

As stated previously, the mice dying or killed were examined for macroscopic evidence of tumor in sites other than that where the dibenzanthracene-lard solution was injected. A number of tumors were found in the lungs, most of which were verified by histological

examination. The number of lung tumors in the various strains is listed below:

Strain A female	18
Strain A male	11
Strain CBA female	1
Strain M male	1
Stock female	3

It is not clear whether these tumors were metastases or primary lung tumors. One lung tumor was observed in a mouse free of tumor at the site of the dibenzanthracene-lard injections. This problem is receiving further consideration.

HISTOLOGICAL FINDINGS

In all, 50 of the 153 tumors arising at the site of injection were examined microscopically. Practically all were spindle-cell sarcomas. While most of the tumors were composed entirely of spindle cells, a few were of the mixed type, containing, in addition to the common spindle cells, considerable numbers of round or of giant cells. One was apparently a mixture of carcinoma and sarcoma. All sections showed active invasion of voluntary muscle. Further evidence of malignancy was obtained from transmission experiments described below.

TRANSPLANTATION EXPERIMENTS

In conformity with the purpose of the experiment, attempts were made to transplant the induced tumors into normal mice. In all, 11 tumors were transplanted by grafts into mice of the same strain as the animal bearing the tumor, as well as into other pure strains or into stock mice. The usual trocar technique was employed in all these experiments. The results are summarized in table 3.

TABLE 3.—*Results of transplantation experiments of dibenzanthracene-lard tumors*

Experiment no.	Strain in which tumor arose	Strains into which original tumor was transplanted																	
		Strain A			Strain C_3H			Strain CBA			Strain M			Strain D			Stock		
		Number of mice inoculated	Positive	Negative	Number of mice inoculated	Positive	Negative	Number of mice inoculated	Positive	Negative	Number of mice inoculated	Positive	Negative	Number of mice inoculated	Positive	Negative	Number of mice inoculated	Positive	Negative
1	A	8	8	0	8	0	8							8	0	8	8	0	8
2	A	3	3	0	6	0	6							6	0	6	6	0	6
3	A	3	3	0	6	0	6							6	0	6	6	0	6
4	A	3	3	0	3	0	3							8	0	5			
5	CBA				5	0	5	5	2	3				6	0	6			
6	CBA				5	0	5	5	3	2									
7	M				7	0	7				7	7	0	7	0	7			
8	M										12	12	0	6	0	6			
9	D				7	0	7							14	14	0			
10	D				4	0	4				8	0	8	15	15	0			
11	C_3H				18	18	0	8	0	8				6	0	6			

The results show clearly that the induced tumors are similar to spontaneous tumors arising within a pure stock, since they grew only in mice of the strain in which the tumor had its origin. No difficulty has been encountered in subsequent serial transmission of two of these tumors into animals of the same strain in which they originated.

SUMMARY

The results of the experiment confirm the findings of Burrows, Hieger, and Kennaway in showing that the subcutaneous injection of dibenzanthracene-lard solution induces sarcomas in mice. In addition, it has been shown that this solution induces tumors in pure-strain mice which, under normal conditions, do not develop spontaneous tumors. Thus, it is shown that the genetic constitution of a pure strain of mice does not prevent the cells from becoming malignant when exposed to this carcinogenic agent.

Transmission experiments demonstrate that the induced tumors grow only in mice of the same strain in which they originated. In this respect they are similar to spontaneous tumors arising in pure-strain mice.

REFERENCE

(1) Burrows, H., Hieger, I., and Kennaway, E. L.: Am. Jour. Cancer, *16* (1932), p. 57.

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A COMPARISON OF THE ACTION OF SOME POLYCYCLIC AROMATIC HYDROCARBONS IN PRODUCING TUMOURS OF CONNECTIVE TISSUE

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INTRODUCTION

The object of the work described here was to investigate the production of tumours of connective tissue by polycyclic hydrocarbons. An earlier paper from this Institute recorded a series of spindle-celled tumours in animals which had received injections of 1:2:5:6-dibenzanthracene (1). In the later experiments recorded below the results obtained with this compound were confirmed, and the action of two other hydrocarbons (5:6-*cyclo*-penteno-1:2-benzanthracene (2, 3), chrysene), and of the medium (lard) used for injection, was examined.

TECHNIQUE

The hydrocarbons were dissolved (2 mg. in 1 c.c.) in the fraction of lard which is liquid at 37° by heating the containing vessel in boiling water; no higher temperature was used at any stage, and the injection needle was not passed through a flame. The material at 37° was injected *sub cutem* in the groin of rats and mice (at first 1 c.c. in rats and 0.5 c.c. in mice weekly; later 3 c.c. in rats and 1 c.c. in mice at longer intervals, regulated by the rate of disappearance of the substance).

PRIMARY TUMOURS AND TRANSPLANTABLE STRAINS

The tumours appeared as solid growths at the periphery of the mass of lard injected; owing to the presence of the latter material it is not possible to fix exactly the time of origin of the tumour. But the dates when the animals died, or were killed on account of the size of the tumours, give the following comparative data.

1:2:5:6-Dibenzanthracene: Of the 20 mice used, 18 died during the first 140 days, and the remaining two before the 210th day. No tumours were obtained in this series. In an earlier experiment (1) the first tumour was obtained on day 144 (average, day 186); hence the negative result of this later series is accounted for by the heavy death rate. Of the two series of 10 rats each, one yielded 7 tumours (Fig. 1), the animals being killed between days 161 and 217 (average, day 193), this last figure being similar to that given by an earlier series (1) (average, day 210). The second series of

TABLE I: *Subcutaneous Injection of Hydrocarbons, and of Lard*

Substance injected	Animals R = Rats M = Mice	Number of Animals in which Tumours Appeared	Day of Death of Tumour-bearing Animals: Range	Day of Death of Tumour-bearing Animals: Average	Transplantable Tumours: Reference Number of Primary Tumour	Transplantable Tumours: Result of Graft	Remarks
1 : 2 : 5 : 6-dibenzanthracene	10R	7	161–217	193	R66	Not carried on beyond 7th gen.	
	10R	1	182				8 died before day 91
	20M	0					18 died before day 140; last 2 before day 210
5 : 6-cyclopentenoo-1 : 2-benzanthracene	10R	8	364–630	422	R117	Failed in 4th gen.	
					R138	Negative	
					R170	Now in 6th gen., 10 per cent takes	
	20M	4	196–280	250	M142	Not carried on beyond 5th gen.	11 died before day 105
					M154	Now in 39th gen., 100 per cent takes	
					M157	Not carried on beyond 27th gen.; then 100 per cent takes	
Chrysene	10R	4	35, 39c, 42c, 62c	149	R118	Negative	
					R119	Failed in 4th gen.	
					R122	Failed in 14th gen.	
	10M	0	—	—	—	—	L.M.D. day 350
Lard	10R	2	385, 462		R113	Negative	L.R.D. day 721
					R141	Negative	
	20M	0	—	—	—	—	L.M.D. day 581

gen. = generation. L.R.D. = last rat died. L.M.D. = last mouse died.

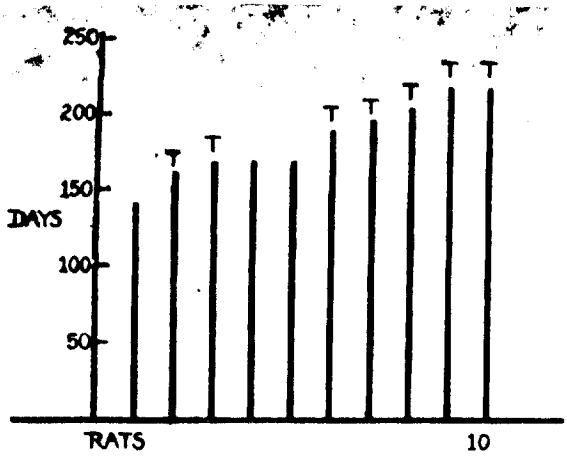

FIG. 1. EXPERIMENTS WITH 1:2:5:6-DIBENZANTHRACENE, IN RATS

In this and Figs. 2 and 3 each vertical line represents the duration of the life of one animal from the beginning of the experiment. T = tumour present at death.

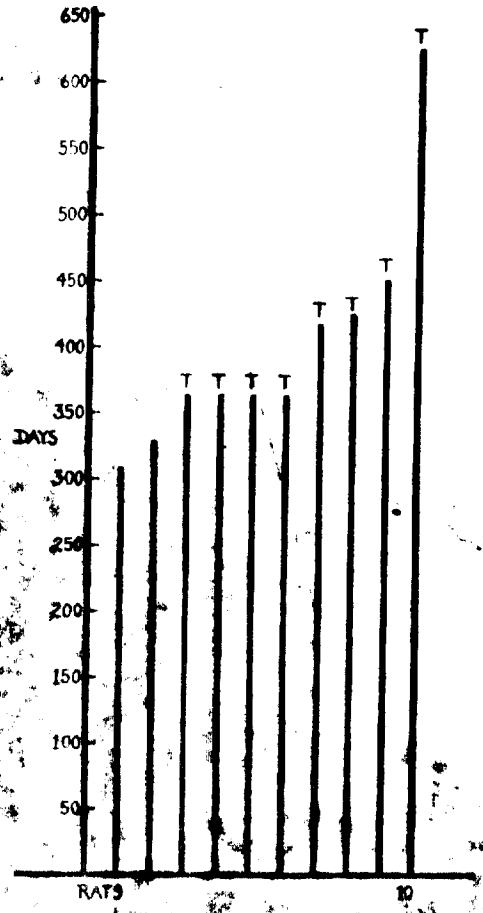

FIG. 2. EXPERIMENTS WITH 5:6 CYCLOPENTENO 1:2-BENZANTHRACENE, IN RATS

ten suffered, as did the mice, from a heavy death rate (8 animals dead before the 91st day), and yielded one tumour only (day 182). A transplantable strain was not carried on beyond the 7th generation (a strain from a tumour of the earlier series (1) in rats is now growing well in the 46th generation).

5: 6-cyclopenteno-1: 2-benzanthracene: The series of rats, and of mice, showed a distinct contrast in time-incidence of tumours; much larger numbers would of course be needed to show if there is any constancy in this (Figs. 2 and 3). Tumours were produced in 8 out of 10 rats (day 364 to 630, average 422) and in 4 out of 8

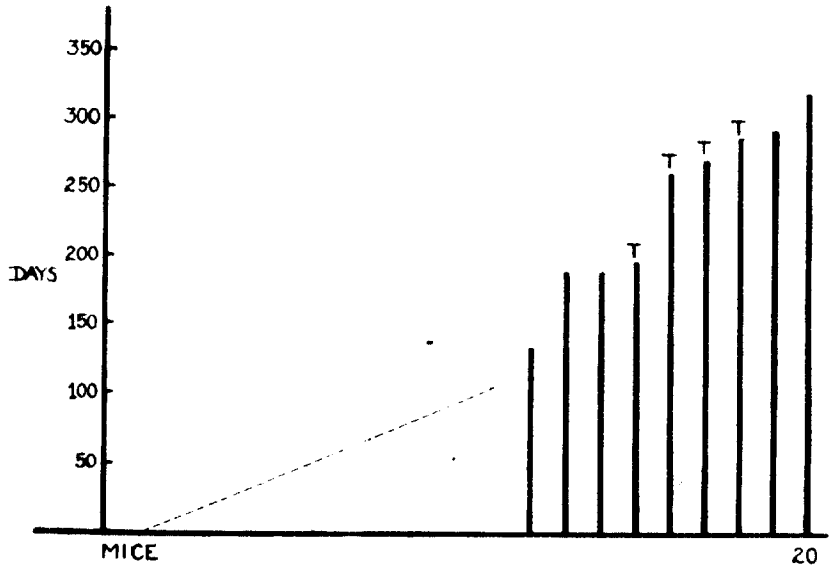

FIG. 3. EXPERIMENTS WITH 5: 6-CYCLOPENTENO-1: 2-BENZANTHRACENE, IN MICE
Eleven mice died before day 105; the duration of life of each of these is not represented separately in the chart.

mice living beyond the 20th week (day 196 to 280, average 250; in this series 11 out of 20 mice died in the first 105 days). This apparent greater susceptibility of mice is shown, also, by the grafted tumours; two out of three attempts failed to establish a strain in rats, while three mouse tumours gave strains of which two grew very freely and for many generations gave 100 per cent of takes (Table I. Fig. 13).

Chrysene: A series of 10 mice yielded no tumours, although 5 lived for more than 280 days. Four out of 10 rats produced tumours (day of death 357 to 626, average 449). Of these tumours, 3 were grafted and 2 gave transplantable strains which have now died out (Table I); that which persisted longest (to the 14th generation) gave in the 2nd generation 9 takes in 10 rats.

Lard: Twenty mice, of which 5 lived for more than 476 days, the last dying on day 581, yielded no tumours. Two rats out of 10 (day of death, 385 and 462) produced tumour-like masses of tissue of which the microscopic structure is described below (Fig. 14). Grafts from both were negative and no metastases could be found. The last rat of the series lived until day 721.

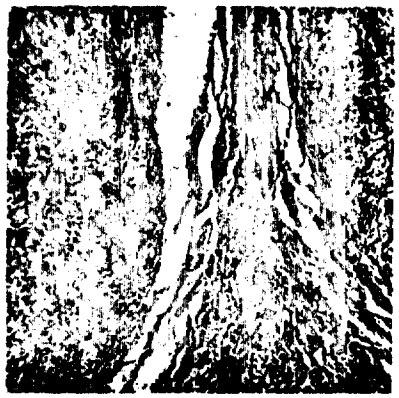

FIG. 4

FIG. 5

FIG. 4. RAT 66, 1:2:5:6-DIBENZANTHRACENE: METASTASES IN GLAND IN RIGHT AXILLA, DAY 151. × 48
Two nodules of spindle-celled tissue (at sides) and lymphoid tissue (in middle).
FIG. 5. RAT 66 (SEE FIG. 4): METASTASIS IN GLAND BESIDE RIGHT URETER (U). × 10

HISTOLOGY

1:2:5:6-Dibenzanthracene and 5:6-cyclopenteno-1:2-benzanthracene: The structure of these tumours is spindle-celled, the individual tumours showing varying amounts of cytoplasm which give either a more cellular or a more fibromatous appearance (Figs. 4 to 12). The individual grafted strains show a persistence of the same characters in successive generations (Fig. 13).

Chrysene: Of the four tumours, two were of the usual spindle-celled type and two of more polymorph character. The metastases in the one case in which these were present were spindle-celled, with a great deal of ground-substance, giving a myxomatous appearance.

Lard: The tumour-like tissue which appeared in two rats was found to consist largely of necrotic matter and granulation tissue, with some spindle-celled areas in which the cells were mostly much less closely packed than those in the tumours following on the injection of hydrocarbons (Fig. 14).

METASTASES

1:2:5:6-Dibenzanthracene: Of the 4 rats which developed metastases, one (R 69) showed a remarkable picture of generalised

secondary growth. This animal was found on day 168 to bear a tumour. Autografts made on two occasions did not take; the animal was killed on day 217. There was a large ulcerated tumour, partly necrotic, at the site of injection, which had extended through the abdominal wall, giving rise to nodules on the peritoneum. The omentum was adherent to the site of the tumour and showed

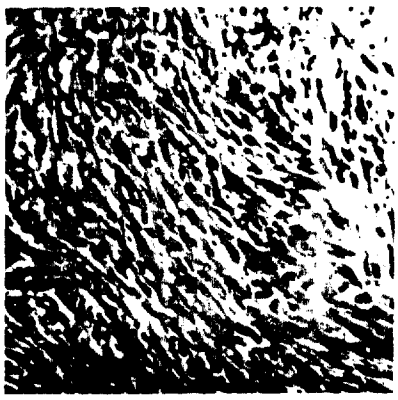

FIG. 6

FIG. 7

FIG. 6. RAT 69, 1:2:5:6-DIBENZANTHRACENE: METASTASIS IN GLAND IN RIGHT AXILLA, DAY 217. × 212

FIG. 7. RAT 69 (SEE FIG. 6): METASTASIS INVADING VOLUNTARY MUSCLE AT COSTAL MARGIN. × 48

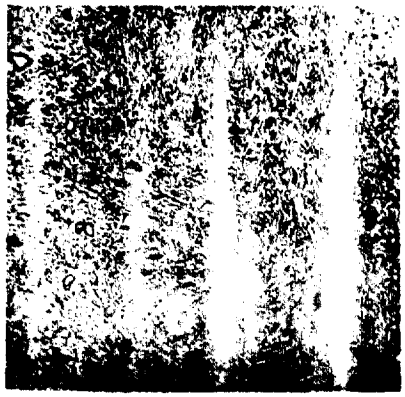

FIG. 8

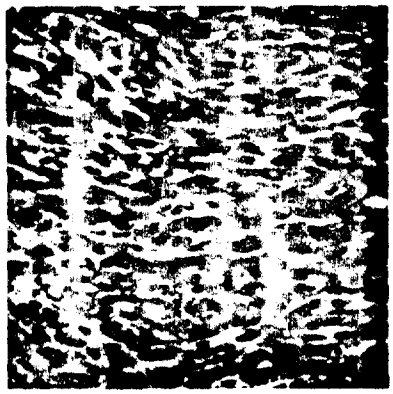

FIG. 9

FIG. 8. RAT 69 (SEE FIG. 6): METASTASIS INVADING PANCREAS. × 55
Note scattered pancreatic alveoli.

FIG. 9. RAT 138, 5:6-CYCLOPENTENO-1:2-BENZANTHRACENE: AUTOGRAFT MADE 28 DAYS BEFORE DEATH, DAY 455. × 212

abundant nodules, some of which were invading the pancreas. There were metastases in the right axillary glands, mesentery, costal margin, lungs, heart and pericardium, and in a lymph gland [illegible] the right ureter (Figs. 6, 7, 8). Metastases in the other

3 rats occurred in the glands in the right axilla (Fig. 4), and alongside the right ureter (Fig. 5), and in the retroperitoneum.

5: 6-cyclopenteno-1: 2-benzanthracene: A rat dead on day 429 showed a spindle-celled primary tumour and multiple secondary nodules on and near the pleural surface of the lung (Fig. 10). A mouse killed on day 259 bore a large secondary nodule attached to the spleen and invading the pancreas (Figs. 11, 12); the primary tumour (M 154) yielded a good transplantable strain which is now giving 100 per cent takes in the 39th generation.

FIG. 10. RAT 139, 5: 6-CYCLOPENTENO-1: 2-BENZANTHRACENE: METASTASES IN LUNG, ONE PROJECTING FROM PLEURAL SURFACE, DAY 429. × 33

FIG. 11. MOUSE 154, 5: 6-CYCLOPENTENO-1: 2-BENZANTHRACENE: METASTATIC NODULE (M) ATTACHED TO SPLEEN (S) AND PANCREAS (P), DAY 259. × 4

Chrysene: One rat showed metastases in the right axillary glands and in the lung (R 19, day 357); a graft from the primary tumour failed in the 4th generation.

AUTOGRAFTS

Autografts were made as follows:

1: 2: 5: 6-Dibenzanthracene	4 rats	No growth
5: 6-cyclopenteno-1: 2-benzanthracene	2 rats	Both grew, one reaching 8 mm. diameter (Fig. 9)
Chrysene	[illegible]	[illegible]

The autografts in the dibenzanthracene series were made very late in the life of the animals, which is perhaps the reason for the failure of growth.

Discussion

Influence of the Fatty Solvent: The results recorded in the section "Histology," above, show that the presence of lard alone in

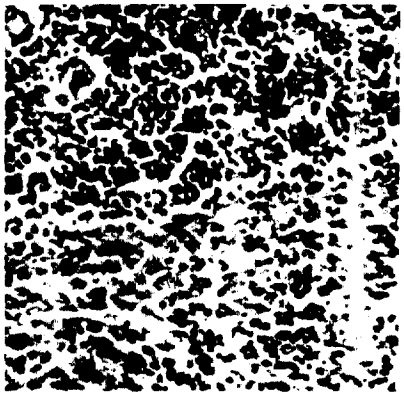

Fig. 12

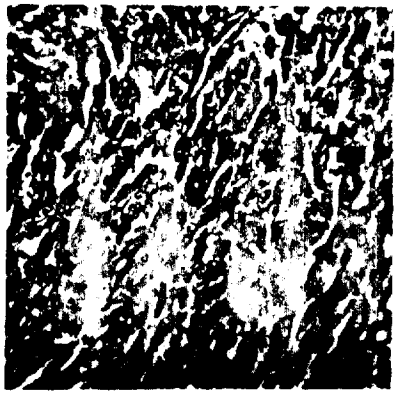

Fig. 13

Fig. 12. Mouse 154 (see Fig. 11): Selfen (above), Tumour (below) In this area capsule of spleen is not yet invaded. × 212.
Fig. 13. Mouse 322, 5: 6 cyclopenteno 1: 2 benzanthracene: 38th Grafted Generation, 15 Days after Inoculation. × 212

the subcutaneous tissue may cause, in a small proportion of animals, a certain amount of neoplastic reaction, with production of some spindle-celled tissue. But malignant characters have not been developed in these experiments by the action of fats alone. We have now a large amount of control data, obtained in the course of these and other investigations, upon the action of fats (chiefly lard and olive oil) injected into the subcutaneous tissue or peritoneal cavity. In this Institute Burrows has made many series of experiments on injection *sub cutem* of unheated lard, and kindly allows us to refer to his results; in a considerable number of animals (88 mice, 145 rats, 8 rabbits, 2 guinea-pigs) no tumours were produced by these means.[1] On the other hand, heated lard, or olive oil, may cause a more active response in the tissue. This is now under investigation here.

Another series of experiments carried out here (by E. Boyland and H. Burrows) has shown that a fatty medium is not essential for the production of malignant tumours of connective tissue by

[1] In an earlier series of experiments upon the injection of lard alone, three tumours appeared, in a series of 130 mice, in one box containing 10 animals only. There was some reason to suppose that an error had occurred here; this has been referred to in an earlier publication (4).

these hydrocarbons. A colloidal solution of 1:2:5:6-dibenzanthracene in water (5), containing no fat, produced spindle-celled tumours in 20 out of 38 rats. A full account of these results will be published shortly.

Duration of Life of the Rat and Mouse: The length of time required for the production of these tumours in the rat and in the mouse does not differ in any constant way which can be attributed to difference in the natural duration of life in the two species.[2] Thus, although the tumours due to 5:6-*cyclo*penteno-1:2-benzanthracene reach full development in a time which is nearly twice as long in the rat as in the mouse (see Table 1), the tumours due to 1:2:5:6-dibenzanthracene show roughly the same time relations in the two species: average days before death: rat (present series) 193; rat (1) 210; mouse (1) 186.

FIG. 14. RAT 141, LARD: SPINDLE CELLED TISSUE, WITH CALCIFIED NODULES, DAY 462. × 48

Question of the Carcinogenic Power of Chrysene: The four tumours obtained in rats with chrysene render it necessary to discuss briefly the significance in carcinogenesis of this hydrocarbon, which has a molecular structure related to that present in the sterol group of naturally occurring compounds.

In the first place it is necessary to emphasize that the chief source of chrysene is coal tar, which contains at least one powerful cancer-producing agent. Moreover, it is extremely difficult to isolate pure chrysene from coal tar; other constituents of the tar adhere very persistently to the chrysene. These facts suggest that the claim made in several publications by Twort and his collaborators (for example, 6, 7) that chrysene is a cancer-producing compound should be regarded with extreme caution. We may point

[2] The evidence on this matter has been reviewed by Wm Cramer, Cancer Review, 7: 241, 1932.

out here that experiments made in this Institute with chrysene have failed to confirm Twort's conclusions.

The chrysene used in our experiments was (i) a sample (A), m.p. 247.5–249.5°, giving a 2:7-dinitroanthraquinone complex, m.p. 295–296.5°, for which we are indebted to Mr. W. Gordon Adam (this has been used for the subcutaneous injections described in this paper), and (ii) a sample (B), m.p. 248–249.5°, giving a dinitroanthraquinone complex, m.p. 298–299°, obtained from the Rütgerswerke-Aktiengesellschaft of Berlin. This latter sample was indistinguishable from pure synthetic chrysene, both in its m.p. and in the bright red colour of the 2:7-dinitroanthraquinone complex.

TABLE II: *Application of Chrysene to Skin of Mice.*

Chrysene Specimen and Solvent	Number of Mice Alive			Day of Death of Last Mouse	Tumours	
	Initial	After 1 year	At present date 3-11-33		Papilloma	Epithelioma
A. 0.3 per cent in benzene	50	24	—	775	1 (day 624)	0
A_1 later B. 7.5 per cent in commercial oleic acid	100	44	1 (day 748)	-	One tumour (in live mouse)	-
B. 0.3 per cent in mouse fat	20	5	2 (day 632)	-	—	—
B. 0.3 per cent in benzene	100	30	27 (day 392)	-	One doubtful tumour (in live mouse)	—
	270	103				

For comparative purposes, two samples of synthetic chrysene have been prepared. One sample, obtained by the method of Weitzenböck and Lieb (8) had m.p. 248.5–249.5°. Another sample, synthesised by a new method, to be described elsewhere, had m.p. 248–249°, and gave a 2:7-dinitroanthraquinone complex, m.p. 297–298°. It is clear from these observations that all the material used in our experiments on animals was of a high order of purity.

Solutions of chrysene in benzene, oleic acid, and mouse fat have now been applied to the skin of 270 mice in all, of which 103 lived for more than one year, with results which are summarised in Table II. It will be observed that no epitheliomata have yet been obtained. Of the three tumours which were formed, one was a papilloma; the other two mice are still living, so that no final opinion can be expressed concerning the malignancy of the tumours which they bear. One series of 100 mice (of which 44 were alive after one year) was treated with a suspension of chry-

sene in oleic acid, exactly as described by Twort and Fulton (6). In this series only one tumour was obtained.

Whether these three tumours, which are all that have arisen in 270 mice, were due to the chrysene or to a trace of very potent impurity must remain undecided until experiments with synthetic chrysene, which are now in progress, have been completed.

Production of Sarcomata and Carcinomata by Hydrocarbons: The connective-tissue tumours described above which appeared after injections of chrysene were obtained in a much higher proportion of the animals (rats) than were the epitheliomata produced by applying chrysene to the skin (of mice). This suggests that the order of carcinogenic potencies of a number of compounds, measured by their capacity to produce sarcomata, is not necessarily that shown by their capacity to produce carcinomata. Such a suggestion also receives support from a consideration of the relative potencies of 1:2:5:6-dibenzanthracene and 5:6-*cyclo*penteno-1:2-benzanthracene. For the latter hydrocarbon appears to be more active in producing epitheliomata (3), whereas the experiments now recorded, in conjunction with those previously reported (1), seem to show that 1:2:5:6-dibenzanthracene is more efficient than 5:6-*cyclo*penteno-1:2-benzanthracene in the production of sarcomata in rats and perhaps also in mice. It has been a matter of some surprise to us that the same agent is able to produce both carcinomata and sarcomata. Possibly further investigations will reveal the existence of substances which are able to produce either sarcomata alone or epitheliomata alone, but not both.

Summary

1. Spindle-celled tumours of connective tissue have been obtained in rats and mice by injection of fatty solutions of 1:2:5:6-dibenzanthracene, 5:6-*cyclo*penteno-1:2-benzanthracene, and chrysene; and a slight amount of neoplastic growth has been produced by the fatty medium (lard) alone. The tumour-like tissue obtained with lard showed none of the characteristics of malignancy.

2. In a series of experiments carried out with chrysene upon the skin of 270 mice, of which 103 lived for more than one year, no epitheliomata have as yet been produced.

3. The same agents (1:2:5:6-dibenzanthracene and 5:6-*cyclo*penteno-1:2-benzanthracene) can produce sarcomata as well as carcinomata, but the order of activity of the two compounds does not seem to be the same for the two types of tumour. This applies both to rats and to mice.

We wish to express our indebtedness to Professor E. L. Kennaway for his constant interest and help during the course of these experiments. Thanks are also

due to Mr. J. Marsh for care of the animals, and to Mr. E. W. Woollard, who has prepared all the microphotographs.

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Identification of Oils and the Detection of Oil Adulteration by Differential Infrared Spectroscopy*

By J. G. BARTLETT and J. H. MAHON (Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Ontario, Canada)

The sophistication of a high-priced food with similar but less expensive products is a problem that has existed for many years. This type of adulteration has been particularly attractive to the unethical supplier of fats and oils because of marked similarity of these substances and the existence of wide price differentials.

Chemically, most fats and oils are enough alike to make the detection of one oil in the presence of another often quite difficult. If the supplier of edible oils blends oils together in such a way that the final product satisfies the conventional analytical constants used as tests of identity (e.g., refractive index, saponification number, iodine number, etc.), adulteration may go undetected. Many specific tests for the detection of one oil in the presence of another depend on non-glyceride constituents. However, modern refining methods that remove these constituents may cause variable results. Because of the large number of possible adulterants, a reasonably complete check on a sample of oil requires the performance of many tests, and hence represents, in most instances, a tedious and time consuming operation. Thus methods of analysis that are rapid, reliable and have a wide application would be very valuable.

A recent problem in this laboratory involved the detection of rapeseed oil in olive oil. Satisfactory chemical tests have been devised (1), but because of the large number of samples involved, a more rapid procedure was sought. While infrared spectroscopy has been widely used for the rapid identification of organic compounds, it has not been used to identify edible oils, since their spectra are very similar. Infrared spectra of a few oils and fats have been reported (2, 3), and the literature in the fats field has been reviewed (4, 5), but no method using infrared spectroscopy to detect one oil in the presence of another has been published. However, in this laboratory in 1954 Stewart showed differences between fats by differential infrared spectroscopy (13), and a suitable method of using this technique was subsequently developed. In the preliminary report on this method (6), the spectra were recorded by using cell lengths of 1 mm. In this paper most of the spectra were obtained by using 0.5 mm cells that gave much more detail in the 1200 to 1080 cm^{-1} region.

The apparatus used for this investigation was a Perkin-Elmer Model 21 infrared spectrophotometer equipped with matched sodium chloride solution cells of 0.5 and 1.0 mm light path. Hypodermic syringes of 0.5 or 1 ml capacity were used for filling the cells.

Experimental

Preliminary investigation in this laboratory showed that the spectra of rapeseed and olive oils, determined in the usual manner, were similar, as shown in Fig. 1, but that there were minor differences in the curves particularly in the 1200 to 900 cm^{-1} region. In order to emphasize these differences, the technique of differential infrared spectroscopy was tried (7). In this procedure, a sample solution is placed in one beam and a reference sample solution in the other beam of the double beam spectrometer. Under these conditions, the instrument measures only the differences between the two beams and does not give any indication of total absorbance. Thus if the same sample is placed in both beams, a straight line will be obtained, whereas if the samples are different, minor differences in the absorption spectra will be evident as deviations above and below the reference line.

If the absorbance of a sample is high, the recorder, which registers linearly with respect to per cent transmittance, records only small changes with changes of 0.1 or 0.2 absorbance units on the chart. However, by

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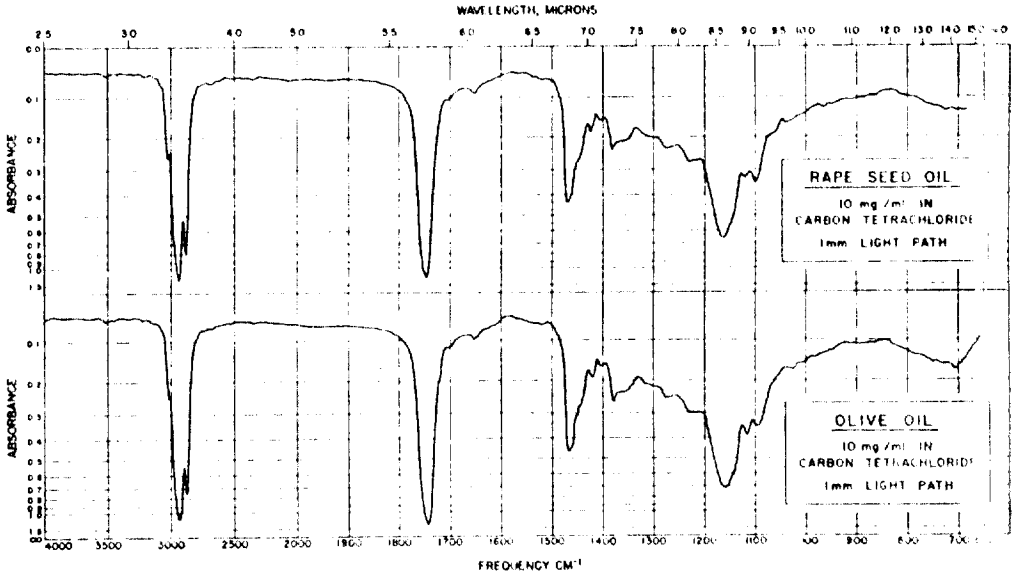

Fig. 1. Infrared spectra of rapeseed oil and olive oil.

using the differential technique, the recording is moved up the chart (to approximately 75% transmission) where the absorbance scale is expanded, and differences of 0.05 absorbance units become apparent. Under these conditions, solutions of high absorbance may be used. Since this technique is essentially the measurement of small differences between two large quantities, we are imposing severe conditions on the apparatus. The cells must be matched as carefully as possible, and the optics of the instrument must be carefully aligned. The amplifier must be stable and free of drift. Even with these precautions, the reference line (i.e., with the same sample in each beam) may show slight deviations from linearity.

Because of the high absorbance of the sample solutions, the recorded differences obtained were not proportional to cell length or concentration since the instrument is unable to record correctly with such low levels of energy. Figures 2, 3, and 4 are differential spectra that illustrate these points. For each curve, a carbon tetrachloride solution of the reference sample of olive oil was placed in the "reference beam" and a solution of a sample consisting of 40% rape seed oil in olive oil was placed in the "sample beam." The spectra were recorded over the

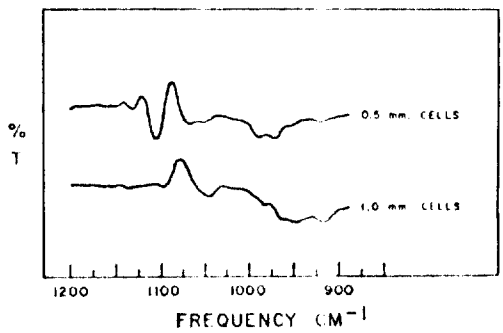

Fig. 2. Effect of cell length on differential spectra of oils.

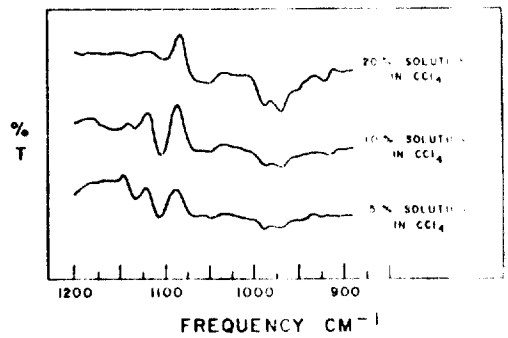

Fig. 3. Effect of concentration on differential spectra of oils.

range 1200–900 cm^{-1}, and except where indicated, 0.5 mm cells, 10% fat in carbon tetrachloride, and a fixed slit of 300μ were used. The beam trimmer balance was set to give a reading of approximately 75% transmission. The effect of cell length is shown in Fig. 2 for 0.5 and 1 mm cells. The region from 1200 to 1075 cm^{-1} is of such high absorbance that the instrument is "dead" when 1 mm cells are used. A carbon tetrachloride band at about 1000 cm^{-1} causes

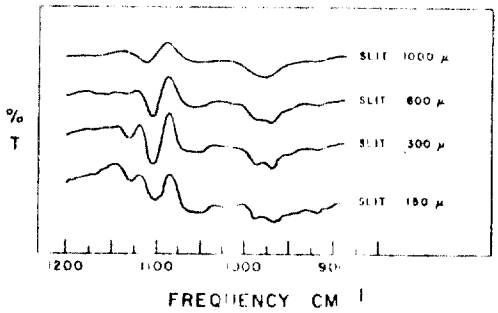

Fig. 4. Effect of slit width on differential spectra of oils.

another "dead" spot with the longer cells. The effect of concentration of oil in the carbon tetrachloride solution is shown in Fig. 3. There is a gain in the differential spectra between 5 and 10% but it is not proportional to the concentration. When 20% solutions are used, the instrument shows little response. The effect of varying the slit is shown in Fig. 4. If an attempt is made to increase resolution with narrower slits the instrument response is poor. When the slits are opened the resolution is reduced and stray light becomes a factor.

In order to use the differential technique, the concentrations of all the samples must be exactly the same so that any differences in absorbance will be due to differences in the spectra, and not reflect differences in concentration of the two samples in the beams. For this reason, oil solutions were prepared accurately on a weight-volume basis. The solvent used was carbon tetrachloride rather than the more volatile carbon disulfide, frequently the solvent of choice. On the basis of these experiments the following procedure was developed.

Procedure

Weigh 2.50 g of an oil sample into a 25 ml volumetric flask and dilute to volume with CCl_4. Prepare a similar 10% w/v solution of olive oil of known purity for reference. Fill two matched 0.5 mm NaCl cells with the reference oil solution and place them in a double beam infrared spectrometer. The following instrumental conditions are used for the Perkin-Elmer Model 21:

Slit	300 μ (fixed)
Response	1:1
Gain	4.8 to 5.2
Filter	out
Frequency range	1200 to 900 cm^{-1}
Prism	sodium chloride
Source current	0.3 amp.
Beam trimmer balance	To give a reading of approximately 75% transmission

Obtain a tracing which will be a measure of the alignment of the instrument and the matching of the cells. This is referred to as the "reference line." Rinse and fill the sample-beam cell with the solution of the oil under examination, and repeat the recording over the same wavelength region without changing instrumental settings or chart paper.

It was found convenient to use pieces of graph paper 8½×11 inches taped to the recorder drum. Pens with different colored inks for the two tracings were of advantage.

Results

The differential spectra that are presented in Figs. 5 to 21 were obtained by using a 10% w/v solution of a Spanish olive oil in the reference beam of the spectrophotometer. The bottom curve of Fig. 7 shows the tracing obtained with this oil in both beams.

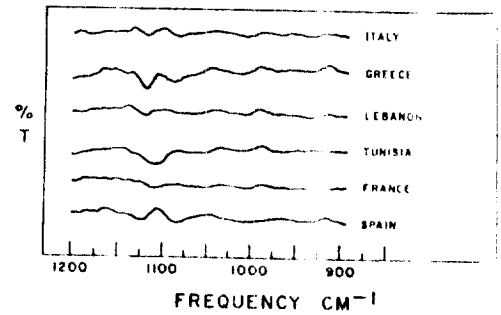

Fig. 5. Differential spectra of olive oils from various sources.

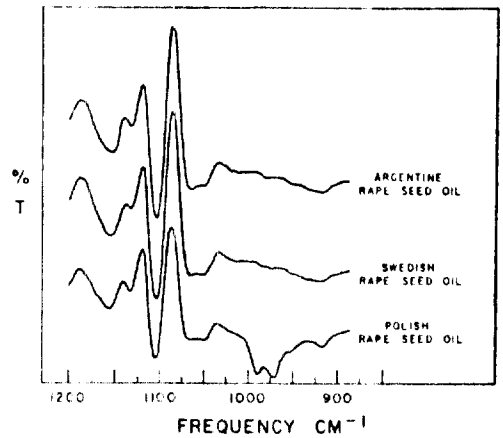

Fig. 6. Differential spectra of rapeseed oil.

and is the curve referred to as the "reference line" in the procedure. In these figures the per cent transmission scale has been displaced vertically for each curve in order to display more than one spectrum in each figure. In most cases the curves would coincide at 890 cm^{-1}. In some spectra the deviations were sufficient that the pen was driven to scale limit, as indicated by flat tops to some peaks. Peaks upward may be considered as negative peaks since they represent regions in which the absorbance is less than that of the reference, while peaks downward are considered as positive.

(*a*) *Differential Spectra of Olive Oil.*—Minor deviations, particularly in the neighborhood of 1100 and 1050 cm^{-1}, were encountered with olive oils from different sources. This procedure has been used to check 252 samples of olive oil from 12 sources. Representative spectra are shown in Fig. 5. The deviations from the reference line at 1125 to 1100 cm^{-1} appear to be due to the natural range of concentration of unsaturated fatty acids encountered in olive oil.

(*b*) *Differential Spectra of Rapeseed Oils and Olive Oil-Rapeseed Oil Mixtures, with Olive Oil as Reference.*—Since adulteration of olive oil with rapeseed oil was suspected, differential spectra of several different types of rapeseed oil and rapeseed-olive oil mixtures were recorded over the range 4000 to 850 cm^{-1}. Differences between the oils were noted at 3100, 1750, and 1400–1300 cm^{-1}, but the most striking features of the differential spectrum were negative peaks at 1130 and 1080 cm^{-1} with a characteristic contour from 1200 to 900 cm^{-1}, which persisted in mixtures containing as little as 10% rapeseed oil. Figure 6 shows the curves obtained from different types of rapeseed oil containing from 25 to 45% erucic acid. The effect of

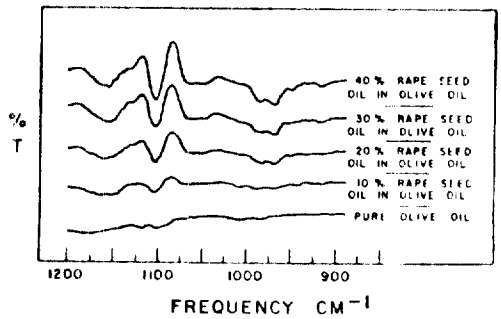

Fig. 7. Differential spectra of rapeseed-olive oil mixtures.

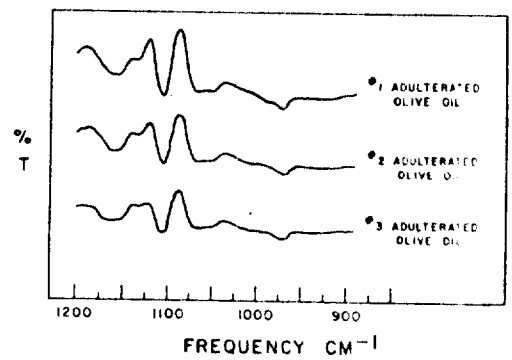

Fig. 8. Differential spectra of olive oil samples adulterated with rapeseed oil.

the addition of rapeseed oil on the iodine number, refractive index, and saponification number of a typical olive oil is given in Table 1. The indicated values give the level of adulteration necessary to bring each of these constants significantly outside the range allowed for olive oil in the Canadian Food and Drug Regulations. Figure 7, which presents the spectra of such mixtures, shows that the infrared procedure is a more sensitive test for adulteration with rapeseed oil since as little as 10% may be detected. Differential spectra of a number of olive oil samples from one supplier showed the characteristics of rapeseed oil adulteration to the

Table 1. Typical analyses of olive-rapeseed oil mixtures

Olive Oil, %	Rapeseed Oil, %	Refractive Index, 20°C	Saponification Number	Iodine Number
100	0	1.4691	189.8	84.8
90	10	1.4696	189.3	88.0
80	20	1.4702	188.1	89.8
70	30	1.4706[a]	186.3	92.5
60	40	1.4710[a]	185.3	95.3[a]
50	50	1.4714[a]	183.7[a]	97.8[a]
Can. F. & D. Limits		1.468–1.470	185–195	77–94

[a] Values significantly outside the limits of the Canadian Food and Drug Regulations.

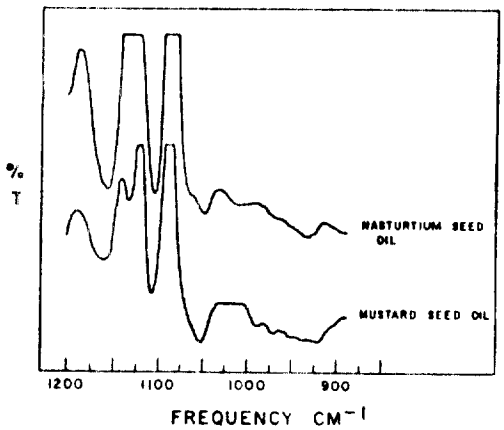

Fig. 9. Differential spectra of oils containing erucic acid.

extent of 25 to 60%. Figure 8 shows the spectra of three of these samples; their analytical values are listed in Table 2.

Nasturtium seed and mustard seed oils, which resemble rapeseed oil in that erucic acid is a major constituent, give differential spectra similar to but not identical with that of rapeseed oil, as shown in Fig. 9.

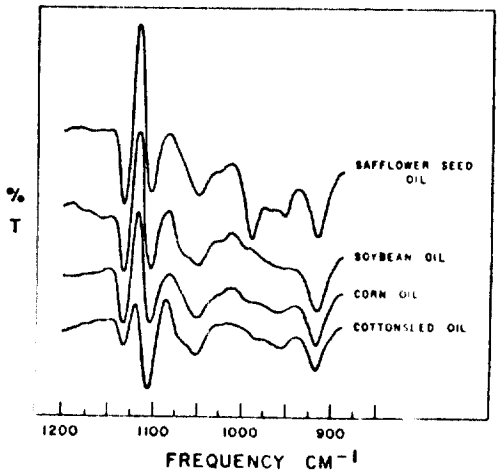

Fig. 10. Differential spectra of vegetable oils.

Table 2. Refractive index, saponification number, and iodine number of commercial olive oil samples adulterated with rapeseed oil

Sample	Refractive Index, 20° C	Saponification Number	Iodine Number
1	1.4709[a]	185.7	95.7[a]
2	1.4704[a]	186.8	91.6
3	1.4702	188.4	93.6
Canadian F & D Limits	1.468–1.470	185–195	77–94

[a] Values significantly outside limits of Canadian Food and Drug Regulations.

(*c*) *Differential Spectra of Vegetable Oils with Olive Oil as a Reference.*—In addition to rapeseed oil, about 30 other vegetable oil spectra have been obtained. The differential spectra with reference to olive oil of some commercially important oils are shown in Figs. 10 to 12 and 20% mixtures of some of these oils with olive oil in Figs. 13 and 14. In addition to the differential spectra presented here, the spectra of a number of other oils have been obtained over the region of 1200 to 850 cm^{-1} in order to permit assignment of spectral characteristics to known constituents. These spectra are not illustrated but the oils studied are listed in Table 3.

(*d*) *Differential Spectra of Animal Fats and Oils with Olive Oil as a Reference.*—While it is not anticipated that animal fats will be used as adulterants for olive oil, the differential spectra of a number of fats of this type have also been obtained in order to complete the collection of spectra for identification of fats and oils by differential spectroscopy. Six of these spectra are shown in Figures 15 and 16; other fats also tested are listed in Table 3.

Table 3. Oils and fats studied by the differential infrared spectroscopy procedure

Sample	Oil or Fat	No. of Samples	Figure
	A: Vegetable Fats		
1	bayberry[a]	1	
2	boxwood	1	
3	castor	1	
4	coconut	2	11, 21
5	corn	2	10, 14
6	cotton seed	2	10, 14
7	cyperus	1	
8	delphinium seed[c]	1	
9	ergot	1	
10	grapeseed	1	
11	gymnocladus	1	
12	jojoba[b]	1	
13	kenaf	1	
14	linseed	3	
15	mustard seed	1	9
16	nasturtium seed	1	9
17	okra	1	
18	olive	252	1, 5, 17
19	palm	2	11
20	palm kernel	2	11
21	peanut	1	12
22	rapeseed	6	1, 6, 7, 8, 21
23	rice bran	1	
24	safflower seed	2	10, 13
25	sesame	3	12, 14
26	soybean	4	10, 13, 18
27	sunflower seed	1	12, 13
28	tea seed	1	12
29	tung	1	
	B: Animal Fats		
30	butter	29	15
31	herring	3	16
32	lard	4	15
33	menhaden	1	16
34	muskox	1	
35	polar bear	1	
36	rat kidney	5	
37	seal	3	16
38	sperm whale[b]	2	
39	tallow	3	15
40	whale	1	
	C: Hydrogenated Fats		
41	coconut	1	
42	cotton seed	1	20
43	herring	2	
44	margarine	5	
45	palm	1	20
46	palm kernel	1	
47	soybean	4	19
48	seal	3	19

[a] Contained significant amounts of free fatty acids.
[b] Fatty alcohol esters—waxes, not glyceride fats.

(c) *The Effect of Refining and Hydrogenation of Oils on Differential Spectra with Olive Oil as Reference.*—A number of samples in different states of refinement and hydrogenation were also examined. Refinement of olive oil causes little change in the spectrum

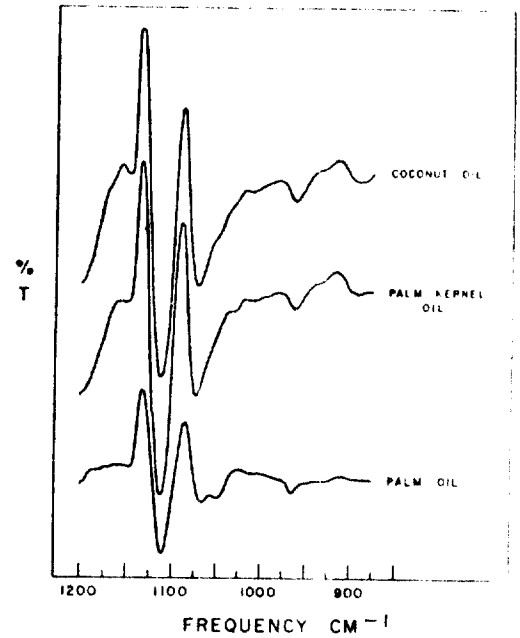

Fig. 11. Differential spectra of vegetable oils

obtained, as illustrated by Fig. 17. However, with soybean oil, the positive peak at 1050 cm^{-1} encountered with the unrefined oil is noticeably reduced by refinement, as shown in Fig. 18.

The effect of hydrogenation was studied with several types of oil. In all cases the positive peak at 912 cm^{-1} was eliminated and the positive peak at 970 cm^{-1} due to a trans double bond was increased, as shown

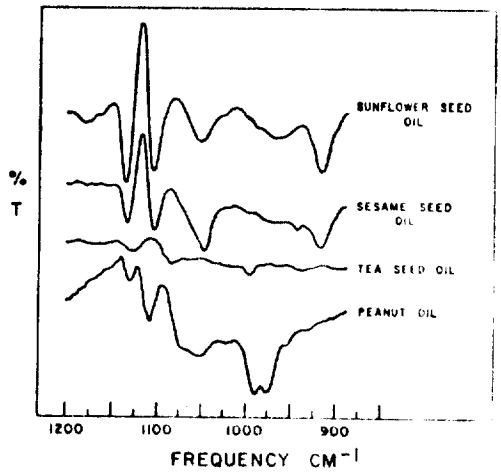

Fig. 12. Differential spectra of vegetable oils.

in Fig. 19. The negative peak at 1100 and 1080 cm^{-1} was gradually increased as hydrogenation became more complete. With fully hydrogenated cotton seed oil and hydrogenated palm oil, the trans double bond peak at 970 cm^{-1} has virtually disappeared, as shown in Fig. 20.

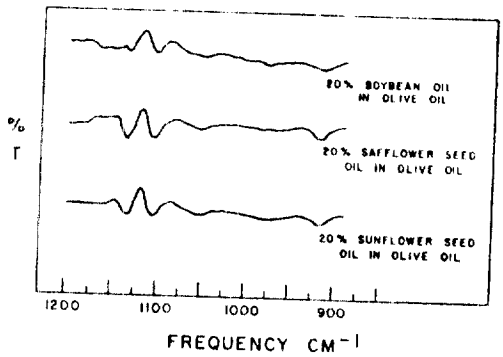

Fig. 13. Differential spectra of vegetable oil-olive oil mixtures.

(*f*) *Differential Spectra of Rapeseed-Coconut-Olive Oil Mixtures with Olive Oil as Reference.*—A mixture containing rapeseed and coconut oils in the proportion of 4:1 has an iodine number, saponification number, and refractive index close to the range of pure

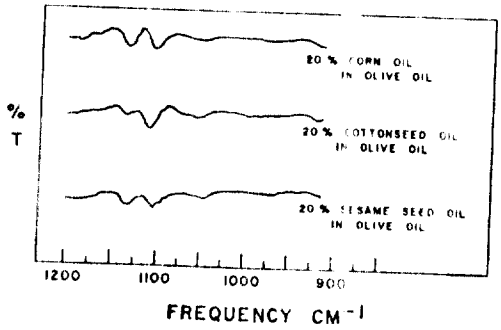

Fig. 14. Differential spectra of vegetable oil-olive oil mixtures.

olive oil. Thus, if this mixture were added to olive oil, the use of these constants would not detect this adulteration. However, the presence of such a mixture is detected spectrophotometrically even at the 10% level of adulteration. The differential spectrum for a 20% mixture is shown in Fig. 21.

Discussion

(*a*) *Applications of Differential Infrared Spectroscopy to Fats and Oils.*—The time required to obtain the differential spectrum of an oil sample is about 10 minutes. For this reason, the procedure is one of the fastest techniques available for checking the purity of an oil sample. Since about 10% to 20% of most fats and oils can be detected in olive oil by this one test, considerable effort can be saved by the elimination of the many tests now necessary to check olive oil for each possible adulterant. Of the oils tested only tea seed oil showed a spectrum which

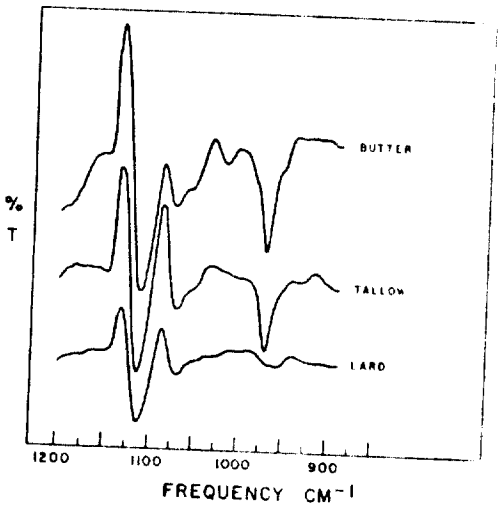

Fig. 15. Differential spectra of animal fats.

was similar to olive oil and would not be detected by this procedure. However, there is a specific color test for tea seed oil (8). An extension of this technique to other oils should be possible for the detection of adulteration. Preliminary tests have shown promise for the detection of lard, tallow, and hydrogenated fats in butter fat.

Since each of the oils tested has a characteristic spectrum, it will be possible to make a rapid identification of many unknown oil samples. Oils that are similar to each other in character, such as soybean, corn, and cottonseed oils, have differential spectra with respect to olive oil that are very similar. However, if one of these is used for reference, differences between these oils become

apparent. Thus, an unknown fat or oil may easily be identified, at least within a group, by differential spectroscopy with olive oil as reference. More positive identification can then be made by obtaining the spectrum of the unknown with one of this group as reference.

Preliminary tests have shown differences in rat kidney fat due to different dietary sources of fat. Kidney fat from rats fed on corn or rapeseed oils showed deviations from that of rats fed on olive oil. These deviations were similar to the differences in the original oils.

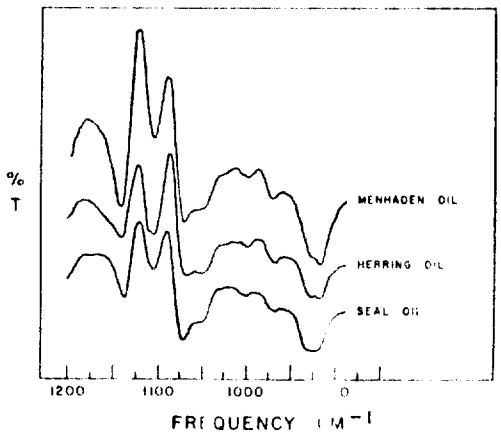

Fig. 16. Differential spectra of marine animal oils.

This technique has also been applied to essential oils and will be the subject of another report (9). The possibility of extending this procedure to flavor extracts is being examined.

(b) Assignment of Spectral Bands.—It is not the purpose of this report to correlate the differential spectra with specific vibrations in the oil molecule. However, an attempt is made to relate the spectra to the composition of the individual oils.

Prominent differences in the spectra of many of the fats and oils tested occur between 1130 and 1070 cm⁻¹. In the normal spectrum there is a peak at 1112 cm⁻¹ (9.0 μ). With relatively saturated fats such as palm kernel and coconut oils, the absorbance is highest and the peak is sharpest. When a fat contains a large proportion of unsaturated acids, this absorbance decreases and the peak broadens or may split into two minor peaks at about 1120 cm⁻¹ and 1100 cm⁻¹. A good correlation was obtained between the total of acids unsaturated at the

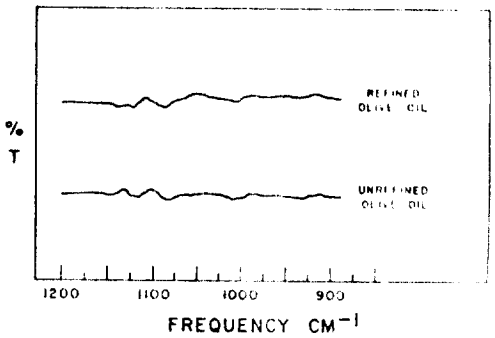

Fig. 17. Effect of refining on differential spectrum of olive oil.

C_9 position and the difference in peak heights at 1112 and 1130 cm⁻¹, by taking measurements from the differential spectra with respect to olive oil, and making corrections

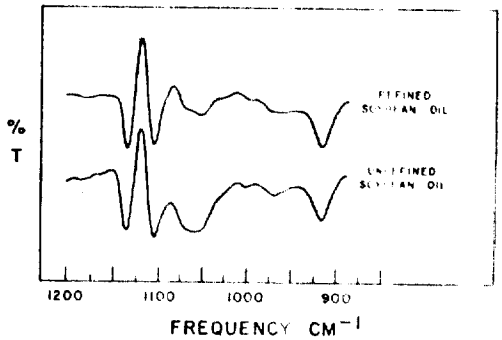

Fig. 18. Effect of refining on differential spectrum of soybean oil.

tions for the molar concentration of the fats. However, it was apparent from the results that linoleic and linolenic acids in fats had about 1.5 times the influence of oleic acid on the peak at 1112 cm⁻¹ and that erucic acid (in nasturtium seed, rapeseed, and mustard seed oils) with a double bond at C_{13} has only about 0.5 times the influence of oleic acid. Using these factors, the difference in peak height at 1130 and 1112 cm⁻¹ was plotted

against % oleic + 1.5 × % linoleic + 1.5 × % linolenic + 0.5 × % erucic acids, as illustrated in Fig. 22. In this figure the values for the composition of the oils are taken from the literature and do not necessarily represent the exact composition of the oil samples used. The points are identified by number corresponding to the list of oils in Table 3. The effect of chain unsaturation on the spectrum in this region is clearly illustrated in this figure, since the oils represented vary from the saturated type of coconut and palm kernel oils to the highly unsaturated oils such as safflower seed oil.

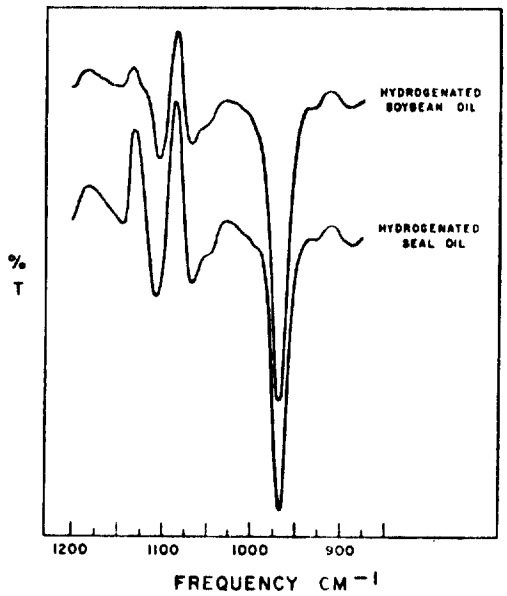

Fig. 19. Differential spectra of partially hydrogenated oils.

Apparently this effect also extends to 1080 cm^{-1}, but the changes are more difficult to analyze, because this region is on the slope of a peak in the normal spectrum. The negative peak observed at 1080 cm^{-1} with rapeseed, coconut oils, etc., in the differential spectra, may be due to a hidden peak in the normal spectrum, or, alternatively, to a shift in peak wavelength. This peak occurs with saturated fats, hydrogenated fats, and, in particular, with oils containing erucic acid (rapeseed oil) or other long chain acids (fish oils).

Fats with short chain-length acids or a low iodine number show a positive peak at about 1065 cm^{-1} that is lacking in oils with significant unsaturation.

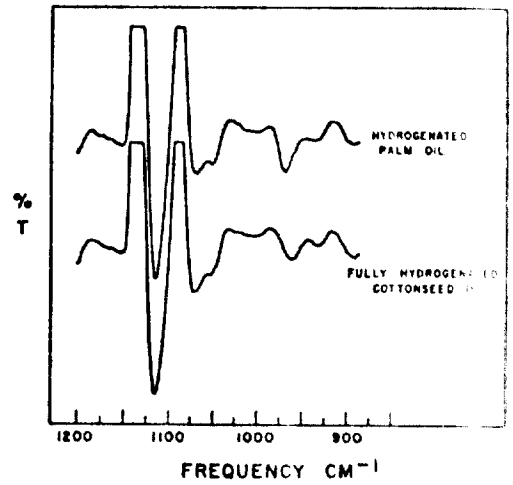

Fig. 20. Differential spectra of hydrogenated fats with low iodine number.

Some oils exhibit a positive peak at 1050 cm^{-1} that has been assigned to the hydroxyl group (2). This peak is present most strongly in the spectra of castor and ergot oils (not illustrated), which contain ricinoleic acid. In some cases, it may also be due to hydroxyl

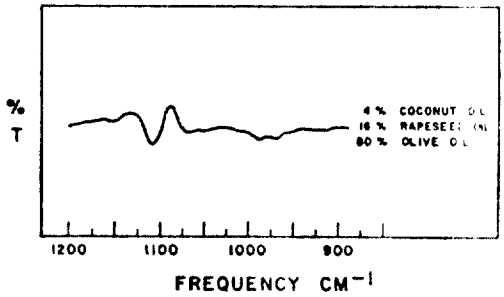

Fig. 21. Differential spectrum of olive-rapeseed-coconut oil mixture.

compounds such as sterols that are minor constituents of the oils. There is a significant decrease in this peak in refined soybean oil as compared to the crude oil (Fig. 18). A cholesterol spectrum, obtained by normal recording, had a strong absorption peak at

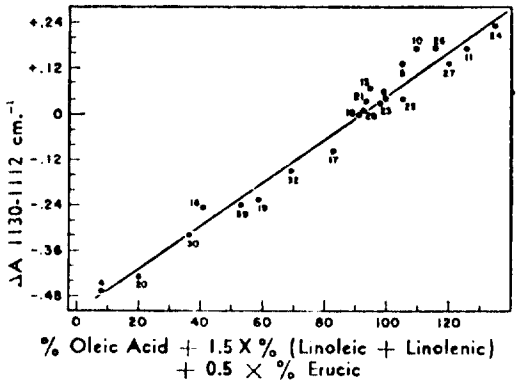

Fig. 22. Effect of unsaturation on differential infrared spectra with respect to olive oil at 1130 and 1112 cm.$^{-1}$

this wavelength. Some olive oils, which exhibited a slight peak at this wavelength, showed no peak upon drying with silica gel, indicating that traces of water may also be responsible for this peak in some samples.

A positive peak at 990 cm^{-1} is present in the spectra of tung oil, safflower seed oil, and some other unsaturated oils. Apparently, it is also related to unsaturation in the fatty acid chain.

The positive peak at 970 cm^{-1} in the spectra of hydrogenated oils and butter has been assigned to the trans-substituted ethylene structure (10).

Oils with a higher iodine value than olive oil show a fairly strong positive peak at 912 cm^{-1}. This appears to be due to multiple unsaturation of the fatty acid chain. This peak is visible in spectra, published by Sinclair, *et al.* (11), of the methyl esters of linoleic, linolenic, and arachidonic acids, but is not present in the spectra of methyl oleate or elaidate.

The spectra of some unrefined oils show a broad positive peak at 940 cm^{-1} that is attributed to the carboxylic group of free fatty acids (12). Delphinium seed oil, which contained about 50% free fatty acid, had a very high absorbance at this frequency.

Acknowledgments

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GASTRO-PAPILLOMATOSIS DUE TO VITAMIN A DEFICIENCY INDUCED BY HEATED FATS*

BY

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AND

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Since Fibiger (1913) first described experimentally induced tumours of the fore-stomach of the rat many other workers have investigated this problem without, however, reaching a satisfactory explanation of his original results. Fibiger described three types of lesions: (*a*) multiple papillomata of the fore-stomach; (*b*) squamous carcinoma of the fore-stomach; and (*c*) carcinoma of the tongue. He also obtained a carcinoma in a mouse that was transplantable. These lesions he attributed to the presence of a nematode, *Gongylonema neoplasticum*, derived from cockroaches which were present in the animal-house and which were consumed by the rats in addition to their diet of white bread and water. Passey (1934) drew attention to the fact that such diet must have been deficient in vitamins, and that vitamin A deficiency in the absence of the gongylonema can lead to papillomatosis of the fore-stomach of the rat. Cramer (1937) reviewed experimental work, including his own investigations bearing on Fibiger's results, and concluded that "diet and parasite are in themselves not sufficient to account for the lesions observed by Fibiger, and that there must be yet another factor involved which has not yet been identified." More recently Roffo (1938, 1939) claimed to have obtained a wide range of pathological lesions, including ulcers, papilloma, and carcinoma of the fore-stomach, adenocarcinoma of the glandular stomach, and sarcoma of the stomach and liver, in rats fed with heated or ultra-violet-irradiated cholesterol, and with overheated animal and vegetable fats. It is clear from the copious illustrations to his papers that Roffo has induced marked hyperplasia of the epithelium of both parts of the rat's stomach, amounting in some cases to histological evidence of malignancy, though the carcinomata do not seem to have metastasized to the liver, as might have been expected. The sarcomata, however, metastasized freely. These experiments are therefore of outstanding interest and demand independent investigation. Roffo believes that carcinogenic hydrocarbons are formed from sterols by the action of heat or ultra-violet rays, but, according to Cook and Kennaway (1940), his views on the nature of the chemical changes involved are inadequately supported by his experimental evidence. The presence of carcinogenic hydrocarbons in the food has been shown by Waterman (1939) to be capable of inducing tumours in the fore-stomach of mice.

In this laboratory an examination of a variety of fats has been made, after different conditions of heating, for the possible presence of known carcinogenic hydrocarbons, but nothing having the characteristic fluorescent spectrum of these substances has yet been demonstrated. It seems possible, therefore, that other types of carcinogen may be partly responsible for Roffo's experimental results. The suggestion that ordinary cooking fats may develop carcinogenic properties under the action of heat raises a practical problem which might have a bearing on the incidence of gastric cancer in man. The fats used in Roffo's experiments were apparently heated to 300-400° C., a higher

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† Working under a full-time grant from the British Empire Cancer Campaign.

range of temperature than is ordinarily reached in domestic cooking, but it seemed possible that similar changes might occur progressively in fats repeatedly heated at lower temperatures. Even in times of peace economic conditions habitually forced a large part of the community to use fats repeatedly for cooking, and our experiments were therefore designed to imitate the worst conditions of frying likely to be in daily use, and to test the fats so treated by feeding them to rats and mice.

Experimental

1. A mixed cooking fat (equal parts of cotton-seed oil and whale oil) was heated to 200-220° C. in a closed iron saucepan for twenty to thirty minutes on thirty consecutive weekdays. The total time of heating amounted to about twelve hours. White bread was fried each day in this fat, allowed to cool, and then fed to 50 Norwegian hooded rats, maintained on a basal diet of whole oats, green vegetables, and water, with the addition of about 2 c.cm. of milk per rat three times a week and 2 grammes of carrot once a week after the first two months. When this experiment had been in progress for twelve months 40 rats had died, of which 8 showed multiple papillomata—6 with central ulceration—1 had a single ulcerated papilloma, and 2 showed irregular hyperplasia of the epithelium, all the lesions occurring in the fore-stomach. One rat developed a carcinoma of the lower lip. As in the foregoing experiment the bread was heated as well as the fat, this variable factor was eliminated in subsequent work by heating the fats alone and, after cooling, spreading them on white bread for feeding to the animals, using unheated fat spread in similar amounts as a control. The survivors of this first group of rats were fed in this manner for a further period of seventeen months, with the addition of about 2 grammes of carrot per rat four times a week, for reasons given below.

2. Twenty-four inbred Wistar albino rats were fed on the basal diet, with the addition of pure lard heated on thirty consecutive weekdays to 200-220° C. for twenty to thirty minutes.

3. Twenty-four Wistar rats were fed on the same basal diet, with the addition of cotton-seed oil, heated in the same way.

4. Twelve Wistar rats were fed on the basal diet, with the addition of unheated lard.

5. Twelve Wistar rats were fed on the basal diet, with the addition of unheated cotton-seed oil. (In Groups 2 to 5, after two months, 2 grammes of carrot once a week, and after a further five months four times a week, was added to the diet.)

6. Fifty stock mice were fed on the basal diet, with the addition of repeatedly heated mixed cooking fat spread on white bread, as described in the second paragraph under Group 1. (After two months 0.5 gramme of carrot once a week, and after a further ten months six times a week, was added to the diet.)

Litters of rats were divided between Groups 2 to 5 so that the groups were genetically comparable. During the first year of these experiments symptoms suggestive of avitaminosis-A were observed in young rats, particularly in Groups 2 and 3. The difference between these groups and the controls became more marked as time passed. The animals were smaller and less robust than the controls, had dry fur and slight xerophthalmia, and avoided exercise except in pursuit of food. As the control animals showed no such symptoms it seemed that the addition of heated fat had in some way neutralized or interfered with the metabolism of the dietary vitamin A. This possibility was tested in the following manner:

7. Twenty-four Norwegian hooded rats were fed on the basal diet, with the addition of about 3 c.cm. of milk and about 2 grammes of carrot once a week. This diet cannot be considered deficient in vitamin A. After four days the following additions to the diet were made: (*a*) 6 rats received 0.5 gramme of unheated lard six days a week; (*b*) 6 rats received 0.5 gramme of lard heated at 327° C. for one hour six days a week; (*c*) 6 rats were similarly fed with lard heated at 220° C. for twelve hours; and (*d*) 6 rats were similarly fed with lard heated at 220° C. for one hour. After two months 2 rats from each group were killed and their livers rapidly removed, rinsed in physiological saline, minced, and desiccated. One gramme of desiccated liver was shaken with 5 c.cm. of chloroform for five minutes, the extract was then decanted, and dilutions of 1/1, 1/2, 1/4, up to 1/128, with chloroform, were made in a series of test-tubes. To each tube, containing 0.5 c.cm. of extract, 2 c.cm. of 30% solution of antimony trichloride in chloroform was added, and the highest dilution in which the blue vitamin A colour reaction could be detected was noted. The results of these estimations are shown in Table I.

TABLE I.—*Relative Vitamin A Contents of Rat Livers*

Rat No.	Lard	Vitamin A Content of Liver (Control = 1)
375B 376B	Unheated (control)	1 1
372B 373B	Heated, 220° C. 1 hr.	1 1
363B 366B	Heated, 220° C. 12 hrs.	1/2 1/2
361B 362B	Heated, 327° C. 1 hr.	1/4 1/8

It will be seen that the vitamin A content of the liver was reduced in proportion to the degree and duration of heating to which the lard was subjected. The remaining rats in this experiment were then fed for one month on a diet rich in vitamin A (basal diet, plus 2 grammes of carrot daily); one from each group was sacrificed, and the liver vitamin A estimated in the above manner. No differences were found between them, showing that the interference with vitamin A metabolism due to the heated lard was not permanent. The remainder of the animals are still under observation.

When animals died their stomachs were washed out and then filled with 10% formol-saline and left to fix for twenty-four hours before being opened. Fixation in this way gave very satisfactory preparations, free from distortion. All the stomachs were subjected to independent histological examination by Dr. L. Woodhouse Price, who reported his findings without knowing the details of the experiment. This is the routine procedure with all histological material in this laboratory. All but three lesions classed as papilloma were histologically confirmed. The results are shown in Tables II to V.

TABLE II.—*Lesions in 14 out of 44 Norwegian Hooded Rats, surviving for More than 4 Months, fed on Basal Diet and repeatedly Heated Mixed Cooking Fats (30 Died without Gastric Lesions)*

Rat No.	Duration of Experiment at Death	Lesions
108B	139 days	9 papillomata (5 ulcerated) in fore-stomach
138B	182 ,,	15 ,, (ulcerated) ,,
112B	221 ,,	4 ,, ,, ,,
113B	225 ,,	4 ,, ,, ,,
122B	256 ,,	1 papilloma ,, ,,
111B	256 ,,	1 ,, ,,
127B	268 ,,	21 small papillomata ,,
103B	275 ,,	1 ulcerated papilloma ,,
141B	301 ,,	Marked hyperplasia of fore-stomach
148B	312 ,,	Hyperplasia of fore-stomach
149B	318 ,,	Haemorrhage in fore-stomach
150B	333 ,,	Keratinizing carcinoma of lower lip
128B	354 ,,	2 haemorrhagic ulcers in glandular stomach
105B	436* ,,	Haemorrhage in glandular stomach

* On vitamin-A-rich diet after first year.

TABLE III.—*Gastric Lesions in 6 out of 19 Wistar Rats, surviving for More than 4 Months, fed on Basal Diet plus repeatedly Heated Lard (13 Died without Lesions)*

[illegible]	Duration of Experiment at Death	Lesions
7[illegible]	1[illegible] days	[illegible] ulcerated papilloma in fore-stomach
873	192 ,,	3 ,, ,, ,, ,,
877	209 ,,	2 papillomata (1 ulcerated) ,,
694	301 ,,	4 ulcerated papillomata ,,
713	304 ,,	3 ,, ,, ,,
714	315 ,,	1 papilloma ,,

TABLE IV.—*Gastric Lesions in 3 out of 14 Wistar Rats, surviving for More than 4 Months, fed on Basal Diet plus repeatedly Heated Cotton-seed Oil (11 Died without Lesions)*

Rat No.	Duration of Experiment at Death	Lesions
578	117 days	1 ulcerated papilloma of fore-stomach
969	196 ,,	1 ,, ,, ,,
715	312 ,,	1 ,, ,, ,,

TABLE V.—*Gastric Lesions in 3 out of 45 Stock Mice surviving for more than 4 Months, fed on Basal Diet plus repeatedly Heated Cooking Fat (41 Died without Lesions; 1 Survives)*

Mouse No.	Duration of Experiment at Death	Lesions
1104	271 days	1 papilloma of fore-stomach
1106	279 ,,	1 ,, ,,
1134	385 ,,	Diffuse hyperkeratosis of fore-stomach

In the three groups of rats receiving repeatedly heated fats in addition to the original basal diet 77 survived for more than four months, and 17 of these died within the first year of the experiment and had papillomata in the fore-stomach. Control rats (receiving unheated fats) dying within the same period showed no papillomata, but 2 had minor degrees of hyperplasia. Among 25 old rats (over 2 years) from other experiments 3 showed hyperkeratosis of the fore-stomach, 1 diffuse hyperplasia, and 1 a simple papilloma of the fore-stomach. In our experience similar lesions have not been observed in control rats under 2 years old.

The benzidine test was carried out on stomach contents in cases showing brownish discoloration of the glandular stomach, and was found to be positive, confirming the presence of blood in the stomach cavity, probably derived from small ulcers. Papillomata, ulceration, and consequent haemorrhage appear to be definitely related to the feeding of repeatedly heated fat and associated with symptoms of varying degrees of avitaminosis-A. To test the possible interrelation between these pathological states 10 rats that had been under experiments for a year and 31 for seven months were given a diet rich in vitamin A, consisting of rat-cake nuts (see accompanying formula) daily, carrot 2 grammes four times a week, and about 2 c.cm. of milk once a week, while continuing to receive the differently treated fats as before. Six of these rats died after being on the vitamin-A-rich diet for more than a year, and none of these showed any gross lesion of the stomach. Two survivors are still under observation. The others are accounted for in the tables.

Rat-cake Formula (Supplied by the North-Eastern Agricultural Co-operative Society, Ltd., Bannermill Place, Aberdeen)

Wheat offal (fine middlings No. 2)	19·2%
Ground wheat	19·2%
Sussex ground oats	19·2%
Ground barley	9·5%
Ground maize	9·5%
Meat and bone meal (45% protein)	9·5%
Dried skim milk	7·0%
White fish meal (60% protein)	4·7%
Dried yeast (40% protein)	1·2%
Sodium chloride	0·8%
Cod-liver oil	0·5%

In more recent experiments 92 rats have been maintained for ten months on the vitamin-A-rich diet throughout, with the addition of heated lard (350° C. for one hour or 220° for about twelve hours). No pathological lesions of the stomach have been observed among 10 that have died. *In vitro* tests for vitamin A in mixtures of cod-liver or halibut-liver oil and heated fats dissolved in chloroform were carried out to see whether any direct neutralization occurred, but the usual antimony trichloride blue reaction could not be observed, as the heated fats alone gave a brilliant red colour reaction with this substance. Attempts to separate the vitamin A from the heated fats after mixture were not successful, and it is not certain at present whether any direct destruction of vitamin A by heated fats occurs or whether the vitamin reaction is merely masked by the intensity of the red colour reaction. Further work is being done to clear up these points and to determine the nature of the substance in the heated fat responsible for the red colour reaction with antimony trichloride.

Discussion

The foregoing evidence suggests that there is present in repeatedly heated fats a factor which interferes in some way with the absorption or metabolism of vitamin A. Our results confirm Roffo's claim that heated fats cause papillomatosis in the fore-stomach of rats, but no evidence of glandular tumours or of sarcoma has been found. One rat developed a carcinoma of the lower lip, but the significance of a single case cannot be assessed.

Although no recognizable carcinogenic hydrocarbons were formed in heated fats under the conditions of our experiments, a factor which may provisionally be termed "anti-vitamin A" was produced, and the subsequent pathological lesions developed by rats and mice receiving this factor with an otherwise adequate diet were apparently due to an artificially induced avitaminosis-A. Thus our results correlate those of Cramer and of Passey with some of the results observed by Fibiger and by Roffo. The malignant tumours originally described by Fibiger and the more extensive series of Roffo may have been due to the action of some hitherto unrecognized factors in the presence of avitaminosis-A. Mice were less susceptible to induced avitaminosis-A and papillomatosis than rats.

So far as any application of these experiments to human pathology is concerned there is no evidence of the development in fats, under ordinary cooking conditions, of any known carcinogen, but the anti-vitamin-A factor may possibly be detrimental to health, particularly when the dietary intake of vitamin A is low. If there is any analogy between rats and humans in this respect the addition of vitamin A to the diet in the form of raw carrot seems to be sufficient to counteract the effects of the anti-vitamin. The habit of eating raw carrot seems to be prevalent at the present time, and it is possible that future statistics will show whether this has any influence on the incidence of human gastric diseases.

Summary

Groups of rats and mice were fed with a variety of repeatedly heated fats in addition to an adequate basal diet. Within a year signs of avitaminosis-A appeared among the rats, and cases of ulceration and papillomatosis of the fore-stomach were observed among those that died. Control animals fed with unheated fats and the same basal diet showed no gross pathological lesions. Extracts of the livers of rats fed with lard heated under various conditions in addition to the basal diet contained reduced amounts of vitamin A as compared with controls.

The heated fats did not contain recognizable amounts of any known carcinogen, but they included some substance which interferes with the antimony trichloride reaction for vitamin A, giving a bright red coloration.

Rats fed with repeatedly heated fats in addition to a diet rich in vitamin A (carrot) showed no gross lesions in fore stomach or reduction in liver vitamin A.

The mechanism of papillomatosis in these experiments seems to be related to an induced avitaminosis-A, due to the presence in repeatedly heated fats of an "anti-vitamin A" of undetermined nature.

We wish to acknowledge the valued help given by Dr. L. Woodhouse Price in dealing with a large number of histological preparations, and by Mr. J. G. Chalmers in examining fluorescence spectra of extracts of heated fats.

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EXPERIMENTS IN CARCINOGENESIS: THE EFFECT OF THE SUBCUTANEOUS AND INTRA-PERITONEAL INJECTION OF LARD, OLIVE OIL AND OTHER FATTY MATERIALS IN RATS AND MICE.

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(PLATE LXXII.)

DURING the last few years many experiments have been undertaken at this Institute to test the carcinogenic potency of 1 : 2 : 5 : 6-dibenzanthracene and other compounds when introduced into the subcutaneous tissue or peritoneum of rats and mice. In most of the earlier experiments of this kind the material to be tested was dissolved in lard or olive oil. For purposes of control, lard, olive oil and other fatty materials free from added carcinogenic compounds were injected into rats and mice in the same way; the results of these control experiments are described below.

I. Injections of fats, fatty acids, unsaponifiable materials and liquid paraffin sub cutem *in mice.*

Table I records the completion of control experiments on mice which were mentioned in an earlier paper (Burrows, Hieger and Kennaway, 1932). All the substances named were injected in amounts of 0·2 to 0·5 c.c. *sub cutem* in the groin with the exception of the solutions of oleic and myristic acids (5 per cent. in olive oil) which were injected in the pectoral region. The table includes figures referring to 216 mice from the material dealt with in the present paper and to these we have added the data obtained by Barry and Cook (1934) in this Institute for mice receiving injections of lard, making a total of 236 animals. In no single instance was a tumour produced at the site of injection.

Andervont (1934) injected lard (0·25 c.c. twice and 0·5 c.c. once in 90 days) *sub cutem* in 190 mice and obtained no tumours in 189 days, when the experiment ended, while tumours appeared at the site of injection in 153 out of 368 mice which received during the same period similar injections of lard containing 1 : 2 : 5 : 6-dibenzanthracene. Schabad (1935) injected sunflower oil (0·25 to 0·5 c.c. on three occasions) *sub cutem* in 16 mice and

obtained no tumours in 6½ months, while in mice receiving 1 : 2 : 5 : 6. dibenzanthracene in the same oil sarcomas appeared in 5 out of 15 between the 4th and 8th months.

TABLE I.

Injections of fats, fatty acids, unsaponifiable materials and liquid paraffin sub cutem *in mice.*

Material injected.	Number of mice.		Day of death of last mouse, or of conclusion of experiment.
	Initial.	Survived four months.	
Pig fat	50	21	613
Lard	30	16	410
Unsaponifiable fraction of lard	18	8	577
Oleic acid (technical)	8	3	259
Unsaponifiable fraction of oleic acid (technical)	20	8	337
Olive oil	10	7	540
Linseed oil	10	2	196
Cod-liver oil	10	7	534
Wheat germ oil	10	2 (7 months)	333
Triolein	10	7	540
Oleic acid 5 per cent. in olive oil	10	6	574
Myristic acid 5 per cent. in olive oil	10	3	179
Liquid paraffin	20	9	533
	216	99	...
Lard (Barry and Cook, 1934)	20	10	581

No tumours resulted.

II. Injections of lard and olive oil sub cutem *in rats.*

Care was taken to avoid accidental contamination with any recognised carcinogenic compound, though in most instances the animals were housed in the main animal room. In expts. 3, 6, 15, 16, 19, 20 and 21 (tables II and V) more rigid precautions were exercised; the rats, freshly obtained from a dealer, were placed in new cages and housed in a room in which no animals had previously been kept. All apparatus and utensils used were new and, together with the animals, were retained throughout in the same compartment. The injections were made early in the day, before the operator had engaged in any other work in the laboratories. In addition to these control experiments four have been included in which the injected fat contained theelin (Parke Davis and Co.) (expt. 7), œstrone (expts. 12 and 13) or arsenious acid (expt. 14). The method was to give weekly injections of 1 c.c. until a bulky collection of oil was present under the skin. After this stage had been reached the intervals between the injections were increased, being regulated by the size of the subcutaneous collection of oil. The rats used were from mixed stocks of no special strain.

Among 193 rats given lard or olive oil subcutaneously, eight spindle-celled tumours arose at the site of injection (table II).

TABLE II.

Subcutaneous injections of lard and olive oil in rats.

No. of expt.	Duration in weeks.	Number of rats.	Survived four months.	Material injected.	Site of injection.	Spindle-celled tumour at site of injection.	Period in weeks from first injection to death of tumour-bearing animals.	Other treatment.
1	146	20	17	Lard	Groin	2	79, 146	None
2	99	7	6	Lard	Groin	0	...	None
3	121	10	8	Lard	Groin	1	121	None
4	81	10	6	X-radiated lard	Groin	0	...	None
5	69	10	5	Lard	Groin	1	98	None
6	89	10	7	Lard + 5 per cent. oleic acid	Groin	1	79	None
7	72	7	7	Lard + theelin (Parke Davis and Company)	Groin	1	56	None
8	53	10	7	Lard	Nape	1	53	None
9*	105	20	16	Lard	Nape	0	...	Emulsified olive oil into peritoneum
10	94	30 134	23 102	Lard	Nape	0	...	1 : 2 : 5 : 6-dibenzanthracene into peritoneum
11	80	8	6	Olive oil	Groin	0	...	None
12	71	8	6	Olive oil + œstrone (0·03 per cent.)	Groin	0	...	None
13	48	3	2	Olive oil + œstrone (0·03 per cent.)	Groin	0	...	None
14	106	40 59	28 42	Olive oil + arsenious acid †	Groin	1	97	None
Totals	...	193 193	144 144	...	...	8	...	...

* See also table V.

† Contained 0·0173 per cent. $NaAsO_2$, which gives a concentration of As (0·01 per cent.) of the order of that found in the hyperkeratotic skin of persons liable to arsenic cancer.

Unsuccessful attempts were made to graft the tumours of expts. 3, 6, 7, 8 and 14 into young rats, ten of which were used for each attempt.

A polypus of the uterus was present in one of the tumour-bearing rats of expt. 1. In expt. 12, in which the injected olive oil contained œstrone, mammary tumours appeared in two of the rats (31st and 64th weeks) and a sarcoma of the liver adjacent to a *cysticercus* cyst in another.

A *cysticercus* sarcoma was present also in one of the rats of expt. 14. In expt. 13, in which œstrone was present in the injected oil, lymphosarcomatosis appeared in one of the rats. In expt. 10, in which 1 : 2 : 5 : 6-dibenzanthracene was injected into the peritoneum, 23 of the animals survived for four months or longer and peritoneal tumours appeared in 12 (52 per cent.) of these.

Two of the tumours occurred among the animals treated under the rigid and isolated conditions mentioned above (expts. 3 and 6). In one of the eight instances the tumour formation followed the injection of lard which contained theelin and in another the injection of olive oil containing arsenious acid. These two cases have been included in the tables although it is possible that the theelin or the arsenious acid might have had some part in causing the tumours. Of the 193 animals, 144 survived for periods of more than four months. Tumours are not commonly induced by 1 : 2 : 5 : 6-dibenzanthracene in less than four months, and therefore this period has been arbitrarily chosen when arranging the experimental results in a numerical form, all animals dying at an earlier stage being omitted from further consideration. On this basis the incidence of tumours at the site of injection of lard or olive oil is 5·7 per cent. as compared with 46·3 per cent. after similar injections of lard or olive oil containing 1 : 2 : 5 : 6-dibenzanthracene (table III). It will be observed (eighth column of table II) that the tumours which followed the injections of fat required a long period for their appearance, none developing during the first year. The average time up to the death of the rat in these cases was 93 weeks. Of the total number of rats treated 99 lived for a year or more, and it was in 8 of these that the spindle-celled tumours occurred.

TABLE III.

Subcutaneous or intraperitoneal injections of 1 : 2 : 5 : 6-dibenzanthracene dissolved in lard or olive oil, without other treatment.

Animal.	Number.	Surviving four months.	Tumour at site of injection.	Percentage.	Tumours elsewhere.
Rat . .	50	41	19	46·3	0
Mouse .	10	8	2	25·0	0
Totals .	60	49	21	42·8	0

No spindle-celled tumours arose in any other part of the body in these animals, nor do such tumours commonly develop spontaneously in the groin of rats; it is not easy therefore to escape the conclusion that the tumours were a consequence of the treatment. This would not necessarily mean that lard and olive oil have neoplastic properties themselves. Most of the known carcinogenic compounds are fat-soluble and perhaps it may have been that the artificially established subcutaneous depots of lard or olive oil served to concentrate some neoplastic agent from the body fluids. Another possibility is that the tumours were caused

Plate LXXII

Fig. 1.—Spindle-celled tumour from rat injected with lard (expt. 1). ×430.

Fig. 2.—Spindle-celled tumour from rat injected with lard containing 5 per cent. oleic acid (expt. 6). ×430.

Fig. 3.—Same as fig. 2, showing mammary duct included in tumour. ×155.

Fig. 4.—Spindle-celled tumour from rat injected with lard containing theelin (expt. 7). ×430.

Fig. 5.—Abdominal tumour in mouse following intraperitoneal injections of olive oil (expt. 20). ×430.

Fig. 6.—Mediastinal tumour from same mouse. ×430.

EXPERIMENTAL CARCINOGENESIS

FIG. 1.

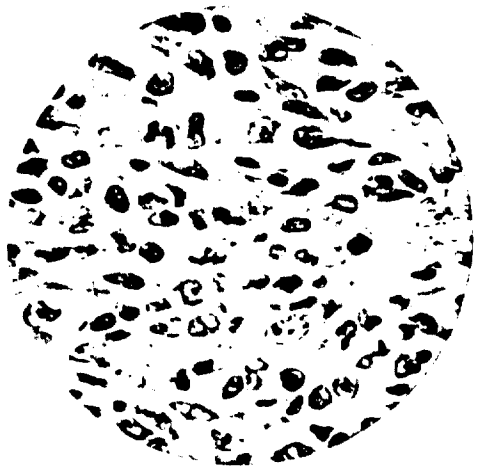

FIG. 2.

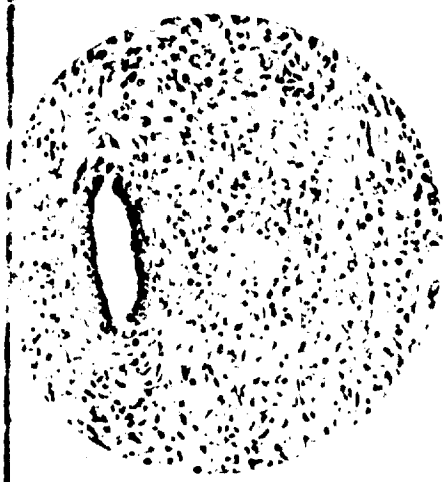

FIG. 3.

FIG. 4.

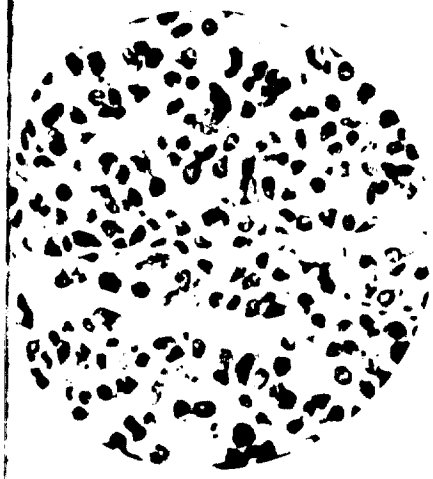

FIG. 5.

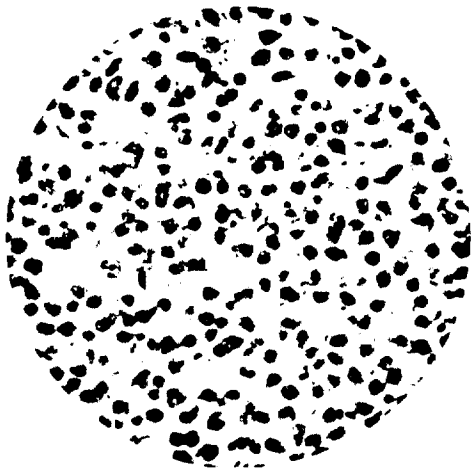

FIG 6.

by " chronic irritation " produced by the lard or olive oil, for the opinion is still held in some quarters that cancer may result from chronic, non-specific irritation.

Nature of the tumours :—When comparing the effects of injecting 1 : 2 : 5 : 6-dibenzanthracene dissolved in lard or olive oil with those produced by injecting the same fatty solvents without the 1 : 2 : 5 : 6-dibenzanthracene, the relatively long periods required for the appearance of neoplasia in the latter series should be recognised.

The tumours which followed the injections of lard or olive oil consisted of spindle cells (figs. 1, 2, 3 and 4), were firm to the touch, grew to a large size, eventually invaded surrounding structures, underwent central necrosis, ulcerated through the skin and killed the hosts. On the other hand no metastases were observed, and the tumours when grafted into other rats failed to grow in every case. Such attempts were made with the tumours occurring in expts. 3, 6, 7, 8 and 14, 10 young rats being grafted on each occasion without any of the grafts becoming established. Considered together, the characters of these tumours suggest that they may represent a low grade of malignant neoplasia. In certain details they differ from the frankly malignant tumours induced by 1 : 2 : 5 : 6-dibenzanthracene and other carcinogenic compounds produced in these laboratories. Tumours induced by the latter, in contrast with those which have followed injections of lard or olive oil only, show more rapid invasion of neighbouring structures, produce metastases, and can generally be transmitted by grafting.

Spindle-celled tumours arising at the site of local injections of lard have already been reported from this Institute. Barry and Cook have described two examples occurring in a batch of 10 control rats given subcutaneous injections of lard alone. The rats died in the 55th and 66th week of the experiment. The tumours consisted largely of necrotic matter and granulation tissue, with some spindle-celled areas. No metastases were found and grafts made from these tumours did not become established.

Special reference should be made, perhaps, to expt. 7, in which a spindle-celled tumour developed in the 56th week at the site of injection of lard containing theelin, for Cori (1927) has recorded an example in which a sarcoma arose in a mouse at the site of injections of ovarian hormone dissolved in " oil " (see addendum).

III. *Injections of heated lard* sub cutem *in rats and mice.*

Some experiments were carried out with heated fat (table IV) in view of the results obtained in this and in other laboratories upon the production of carcinogenic substances by heat. Lard was heated in a metal bath for two hours at 340-360° C. in a stream of nitrogen. The product was shaken with petroleum ether and

watery Na_2CO_3 solution: (a) the petroleum ether extract was washed with water, the solvent distilled off in CO_2 and the residue injected (series 6); (b) the alkaline extract was acidified and the precipitated acids washed with water and mixed with lard (1 : 3 of lard) for injection (series 6A).

TABLE IV.

Injections of heated lard sub cutem *in rats and mice.*

	Series 6.	Series 6A.
Initial number (rats)	9	5
,, ,, (mice)	7	5
Alive after 1 year (rats)	6	5
,, ,, 1 ,, (mice)	4	5
Spindle-celled tumour in one rat	Day 608	Day 509
Last rat dead	Day 713	Experiment stopped, day 588
Last mouse dead	Day 497	Experiment stopped, day 609

On the 608th day a large tumour at the site of injection in a rat of series 6 was autografted and grafted. On the 616th day the rat (L.R. 175) died; the tumour showed spindle-celled areas but was mostly of a more fibromatous character than are the great majority of the tumours produced in connective tissue by carcinogenic hydrocarbons. On the 509th day a spindle-celled tumour of a more cellular character was found at the site of injection in a rat (L.R. 162) of series 6A, and was grafted. No metastases were found accompanying either of these tumours, and the grafts proved negative. Another rat (L.R. 261) of series 6A produced a densely fibrotic tumour containing hardly any cells at all. The mice in these experiments yielded no tumours.

IV. *Intraperitoneal injections of lard or olive oil in rats and mice.*

Repeated intraperitoneal injections of lard or olive oil were made in 60 rats and 80 mice (table V). At first these injections were given once a week, and later at gradually lengthening intervals. No spindle-celled tumours appeared in this series, but peritoneal tumours developed after injections of olive oil in one rat (27th week) and two mice (46th and 62nd weeks). In all three the tumours were multiple and consisted of cells with round, deeply staining nuclei and relatively little cytoplasm (figs. 5 and 6). The cells were supported by a delicate reticulum. There was widespread enlargement of the lymph glands with abundant perivascular collections of round cells in the lungs, liver, kidneys and other organs. In the mice large masses of growth were present in the mediastinum and were invading the pericardium and other structures. Leucæmia or advanced

TABLE V.

Repeated intraperitoneal injections of lard and olive oil.

No. of expt.	Duration in weeks.	Animal.	Number of animals.	Material injected.	Peritoneal tumours.	Period in weeks from first injection to death of tumour-bearing animals.	Other treatment.	Remarks.
9*	105	Rat	20	Olive oil	1†	27	Injections of lard into nape	Multiple tumours
15	89	Rat	10	Lard + 5 per cent. oleic acid	0	...	None	...
16	124	Rat	10	Lard	0	...	None	1 mammary tumour, 124th week
17	114	Rat	20	Olive oil	0	...	None	...
18	60	Mouse	20	Emulsified olive oil	1†	46	None	Multiple tumours, leucæmia
19	43	Mouse	20	Olive oil	0	...	None	...
20	64	Mouse	20	Olive oil	1†	62	None	Multiple tumours, including large mediastinal tumour invading œsophagus. 10 mice grafted: no takes
21	40	Mouse	20	Lard + 5 per cent. oleic acid	0	...	None	

* This experiment is also scheduled in table II (no. 9).

† Round-celled tumours.

leucocytosis was also present in one of the mice: no blood-counts were made, but abnormal numbers of white cells were visible in the blood in the microscopic sections of the tissues.

Although in these instances new growths were present in the abdomen it seems unsafe to assume that the disease began there or that it was a result of the injections. To establish such a connection a more extended experience would be required. A similar condition of lymphosarcomatosis without obvious leucæmia was seen in one of the rats (expt. 8, see table II) subjected to no other treatment than repeated subcutaneous injections of lard into the nape of the neck.

Summary.

Experiments carried out in this Institute, including those of Barry and Cook, upon the injection *sub cutem* of fats and allied materials have yielded 12 spindle-celled tumours in 217 rats and no tumours in 248 mice.

Repeated intraperitoneal injections of lard or olive oil into 60 rats and 80 mice in no instance caused spindle-celled tumours, but in one of the rats and two of the mice lymphosarcomatosis occurred.

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Addendum.

Since this paper was written, Gardner, Smith, Strong and Allen (1936) have reported the development of spindle-cell sarcomas in all of five mice belonging to a pure cancerous strain (Strong C3H) and treated by large daily doses of theelin followed by weekly injections of œstrone benzoate dissolved in oil.

GARDNER, W. U., SMITH, G. M., STRONG, L. C., AND ALLEN, E. Development of sarcoma in male mice receiving estrogenic hormones, *Arch. Path.*, 1936, xxi. 504.

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UTILIZATION OF FAT BY YOUNG PIGS: FATTY ACID COMPOSITION OF INGESTA IN DIFFERENT REGIONS OF THE DIGESTIVE TRACT AND APPARENT AND CORRECTED DIGESTIBILITIES OF CORN OIL, LARD AND TALLOW

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ABSTRACT

Young pigs of 13 to 22 days of age, weaned at 7 days, were used to determine the digestibilities of corn oil, lard and tallow; the mean corrected digestibilities were 86, 81 and 56%, respectively. At 22 days of age, the animals were slaughtered and the fatty acid composition of samples from the contents of six different regions of the digestive system were examined by gas-liquid chromatography. The contents of the digestive systems of a conventional and a germ-free pig which had been receiving the same diet were also examined. The low digestibility of stearic acid and the high digestibilty of oleic and linoleic acids appeared to be a result of microfloral activity rather than a direct result of the pig's ability to absorb these fatty acids.

RESUME

On a déterminé la digestibilité de l'huile de mäis, du saindoux et du suif sur des porcelets de 13 à 22 jours d'âge, sevrés à 7 jours; les digestibilités vraies moyennes ont été de 86, 81 et 56%, respectivement. A 22 jours les animaux ont été abattus et la composition en acides gras d'échantillons du contenu de six régions différentes du tube digestif a été déterminée par chromatographie gaz-liquide. Les contenus du tube digestif d'un porcelet normal et d'un porcelet axénique ("germ-free") qui avaient reçu le même régime ont été également examinés. La faible digestibilité de l'acide stéarique, ainsi que la haute digestibilité des acides oléique et linoléique, paraît être un artéfact provenant de l'activité de la microflore plutôt que le résultat direct de la capacité du porc à absorber ces acides gras.

INTRODUCTION

There is little experimental evidence of an advantage in feeding high fat diets to early-weaned pigs (10). When high fat diets are fed, the digestibility of the added fat is generally low and feed intake is often reduced, which depresses growth rate. However, sow's milk contains 30 to 40% fat on a dry matter basis (6), and this is well digested (7); therefore, investigations of the factors which influence fat digestibility in the young pig would be useful.

The objectives of the experiment reported here were to measure the digestibility of three fats: corn oil, lard and tallow, and to follow the changes in the fatty acid composition of the ingesta as it passed through the gastro-intestinal tract of the young pig.

MATERIALS AND METHODS

Sixteen Yorkshire × Lacombe piglets from two litters were weaned at 7 days of age and were housed individually in cages which allowed separate collection of urine, feces and waste feed. The temperature of the room was maintained between 25 and 28°C; supplemental heating was provided with infrared heat lamps and lighting was continuous. A male and a female from each litter were assigned to each of the four rations (Table 1). Feed and water were provided *ad libitum* and were replaced with fresh material each day.

A preliminary period of 6 days was allowed for the pigs to learn to eat the experimental diets. The feces voided between the 13th and 15th and between the 20th and 22nd days of age were collected and frozen. These samples

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Table 1. Details of diet formulation

Ingredients	Basal %	Corn oil* %	Lard† %	Tallow† %
Soybean 70% protein concentrate‡	38	38	38	38
Corn starch§	20	20	20	20
Cerelose§	35	25	25	25
Fat	0	10	10	10
Cellulose (Solka Floc)‖	2	2	2	2
Iodized salt	0.5	0.5	0.5	0.5
Calcium phosphate (28% Ca, 22% P)	2.9	2.9	2.9	2.9
Limestone (37% Ca)	0.27	0.27	0.27	0.27
Chromic oxide	0.25	0.25	0.25	0.25
DL-methionine	0.6	0.6	0.6	0.6
Vitamin-mineral supplement¶	0.48	0.48	0.48	0.48
Total	100.0	100.0	100.0	100.0
Composition of diets (determined)				
Protein (N × 6.25) (%)	25.0	25.6	25.8	25.3
Fat (%)	0.9	10.4	10.7	10.9
Dry matter (%)	91.3	92.2	92.5	92.5
Gross energy (cal/g)	4.0	4.5	4.5	4.5

*St. Lawrence Starch Company, Port Credit, Ontario.
†Canada Packers, Toronto, Ontario
‡Skidmore Enterprises, Cincinnati, Ohio.
§Canada Starch Company, Toronto, Ontario.
‖Brown Company, New York, N.Y.
¶The vitamin-mineral supplement supplied the following levels of nutrients per kilogram of diet: vitamin A, 8800 IU; vitamin D_3, 1650 IU; vitamin B_{12}, 33μg; riboflavin, 7 mg; calcium pantothenate, 17 mg; choline chloride, 1210 mg; niacin, 20 mg; folic acid, 1 mg; menadione sodium bisulfite, 2 mg; thiamine HCl, 2 mg; pyridoxine, 2 mg; D-biotin, 200μg; santoquin (Delmar), 63 mg; neomycin, 1.4 mg; manganese, 44 mg; copper, 11 mg; iron, 110 mg; zinc, 110 mg.

were freeze-dried and ground for analysis. Dry matter was determined by heating at 105°C for 24 hours. Chromic oxide was determined by the method of Czarnocki *et al.* (5). Gross energy was determined with an oxygen bomb calorimeter.

At 22 days of age the pigs were killed by CO_2 asphyxiation, and their gastrointestinal tracts were removed immediately. Samples of ingesta were taken from six different regions: the stomach, the first half of the small intestine, the second half of the small intestine, the cecum, the colon and the rectum. Each sample was mixed and frozen. The frozen samples were subdivided for analysis; one part (1 to 5 g of dry matter) was used for lipid extraction and another portion was freeze-dried for dry matter and chromic oxide determinations.

Total fat was extracted with chloroform, methanol and water according to the method of Bligh and Dyer (2). The chloroform extract was made up to 100 ml and an aliquot was taken for fatty acid analysis. The remainder of the chloroform extract was reduced to dryness on a rotary evaporator and the residue was weighed.

Approximately 1 mg of the lipid was taken for fatty acid analysis and was transferred to a 16 × 150-mm screw-capped tube for esterification, and 0.2 mg of behenic acid was added as an internal standard. The solvent was removed under reduced pressure on a rotary evaporator using a nitrogen sweep. Ten drops of benzene and 2 ml of 14% boron trifluoride in methanol were added

and the sealed tube was heated to 70°C for 1½ hours. After cooling to room temperature, 4 ml of water was added and the fatty acid esters were extracted with three successive portions of petroleum ether. This was evaporated in a stream of nitrogen and the residue was transferred to a thin layer plate (silica gel G). Benzene was used as the developing solvent and dichlorofluorescein was used to visualize the fatty acid ester band on the thin layer plate. The band was scraped off and extracted with chloroform, dried in a stream of nitrogen and dissolved in 100 μl of carbon disulfide. A suitable amount (2 μl) was separated on a Barber-Colman series 5000 gas chromatograph, equipped with dual columns and dual hydrogen flame ionization detectors. The stainless steel columns, 150 cm × 2 mm inside diameter, were packed with 15% ethylene glycol succinate on 80–100 mesh Gas-Chrom P and were programmed from 160 to 200°C at 4° per minute.

The fatty acid percent composition of the ingesta in the six regions of the digestive tract was investigated in a germ-free pig (maintained in an isolator) and in a pig maintained under the same conditions as those described for the earlier experiment. Both pigs received a liquid diet (SPF-lac) (Borden Company, 350 Madison Avenue, New York 10017).

RESULTS AND DISCUSSION

The dry matter intake and body weight gain of the lard and tallow groups were significantly below those of the basal and corn oil groups (Table 2). However, in all groups there was approximately a 1:1 ratio of dry matter intake to body weight gain. The reduced feed intake of the lard and tallow diets might have been due to the separation of the fat from the rest of the feed in the stomach, which could have caused a delay in gastric emptying. At slaughter, the stomachs of the animals receiving lard and tallow appeared larger than those of the animals receiving either the basal or the corn oil diets. Large globules of fat had separated and collected in the stomachs of the animals fed lard and tallow; in the case of one pig, 65% of the dry matter in its stomach was fat.

The relatively low digestible energy value of the basal ration was due to its low gross energy content, whereas the low digestible energy value of the tallow diet was due to the low digestibility of the dry matter.

Table 2. Dry matter intake, body weight gain, dry matter digestibility, and digestible energy values of the basal diet and the diets containing corn oil, lard and tallow*

Diet	Dry matter† intake (kg)	Gain† (kg)	Dry matter digestibility 2 weeks (%)‡	Dry matter digestibility 3 weeks (%)§	Digestible energy (cal/g DM) 2 weeks‡	Digestible energy (cal/g DM) 3 weeks§
Basal	2.0	2.0	82	81	3.6	3.6
Corn oil	1.8	1.9	82	80	4.0	3.9
Lard	1.4	1.4	80	80	3.9	3.8
Tallow	1.5	1.6	69	73	3.0	3.3

*Mean of four pigs.
†Nine day period, 13 to 22 days of age.
‡13 to 15 days of age.
§20 to 22 days of age.

Table 3. Utilization of dietary fat: total fat voided from 100 grams of dry feed and apparent and corrected fat digestibilities at 2 and 3 weeks of age*

Fat added	Amount in 100 g of dry feed (g)	2 weeks† Fat in feces (g)	2 weeks† Digestibility Appar. (%)	2 weeks† Digestibility Corr. (%)	3 weeks† Fat in feces (g)	3 weeks† Digestibility Appar. (%)	3 weeks† Digestibility Corr. (%)
None	0	1.2	—	—	1.4	—	
Corn oil	10.3	2.3	80	89	3.2	72	83
Lard	10.6	2.9	75	84	3.7	68	78
Tallow	10.8	6.1	48	55	6.2	47	56

*Mean of four pigs.
†See Table 2.

The amounts of fat voided from 100 g of dry feed consumed was determined for the collections taken when the pigs were 2 and 3 weeks of age (Table 3). The mean fat output of the pigs receiving the basal diet was subtracted from the total fat output of each pig receiving the other three diets to indicate the amount of fecal fat derived directly from the dietary fat. It was assumed that the endogenous excretion of fat was the same for all the pigs. This resulted in corrected digestibility values which were approximately 10% higher than the apparent digestibility values. These values are much lower than those reported by other workers; Sewell and Miller (11) found corrected digestibilities of 94% (corn oil), 86% (lard) and 86% (tallow); Cunningham and Brisson (4) found apparent digestibilities of lard ranging between 84 and 91%. However, Eusebio *et al.* (7) used pigs weaned at 17 days and made digestibility determinations at 25 days of age. They found digestibilities of 54% (lard) and 57% (tallow), this value for lard being much lower than the one found in the present study, while the value for tallow was higher. These large discrepancies between the digestibility values reported by different workers indicate that there are factors affecting digestibility determinations in young pigs which have not been controlled. Thus, more of the factors that cause variability in fat digestibility determinations must be eliminated, or controlled, before reliable fat digestibility values of various fats for the young pig can be tabulated.

When the fatty acid composition of the ingesta was investigated throughout the digestive system (Fig. 1), great changes were noticed. There was some increase in the percentage of stearic acid down to the end of the small intestine, but from there the increase in its level was quite dramatic. Conversely, the percentages of oleic and linoleic acids decreased most markedly in the lower digestive system. These changes in concentrations of the fatty acids with 18 carbon atoms cannot be attributed to preferential absorption, since digestion and absorption of fatty acids occur mainly in the duodenum and jejunum of the small intestine and are considered complete by the time the ingested food has passed through the ileum (3). Therefore, the change is attributed to hydrogenation of the unsaturated fatty acids by the microflora (9). However, in previously reported studies the fecal fat has been considered representative of the end product of digestion, and this failure to recognize

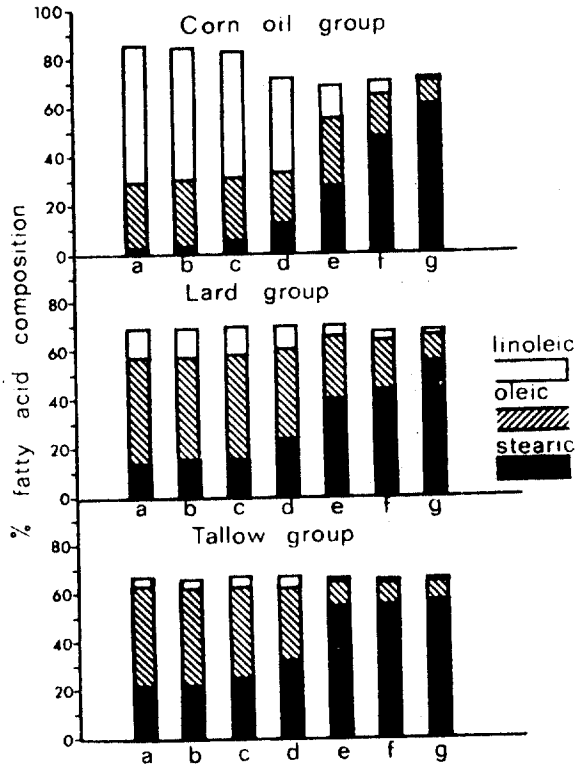

FIG. 1. The levels of stearic, oleic and linoleic acids in the feed (*a*) and ingesta of pigs receiving corn oil, lard and tallow. The six regions of the digestive tract are: stomach (*b*), first half of small intestine (*c*), second half of small intestine (*d*), cecum (*e*), colon (*f*) and rectum (*g*).

the effects of microflora could account for some of the discrepancies between the earlier reports (1, 11) of fatty acid digestibility.

In the present study, the analyses of feed and feces gave very low values for the digestibility of stearic acid and very high values for the digestibilities of oleic, linoleic and linolenic acids (Table 4). It is likely that the wide range in digestibility values obtained for individual fatty acids is not so much a result of the young pig's ability to digest and absorb fatty acids as it is a reflection of the microfloral activity in the lower digestive system.

The chromic oxide indicator tended to separate from the fat in the digestive system, making attempts to determine the absorption of individual fatty acids in the different regions of the digestive system meaningless. This suggests that a fat-soluble indicator would be necessary for quantitative studies of absorption of fat and fatty acids at points within the digestive system.

More evidence of the dramatic effect of the microflora of the lower gut on fatty acid digestibility determinations was obtained from the comparison of the fatty acid compositions of the fat in the digestive systems of the germ-free pig and of the conventional pig, which were both receiving the same diet

Table 4. Fatty acid balance: intake and output (mg/day) from 100 g of dry feed and corrected digestibilities (%)*

Diet:	Basal		Corn oil			Lard			Tallow		
Fatty acid	Input	Output	Input	Output	Corr. dig.	Input	Output	Corr. dig.	Input	Output	Corr. dig.
14:0	1	3	28	3	100	130	10	95	270	6	99
15:0	tr†	16	—	19	—	—	13	—	70	9	110
16:0	58	60	1240	210	88	2760	490	84	2540	370	88
16:1	tr	8	tr	3	400	270	5	101	280	6	101
17:0	tr	12	—	6	—	—	4	—	110	22	91
18:0	25	90	220	690	−170	1570	840	52	2210	810	67
18:1	102	20	2780	120	96	4620	230	95	4130	120	98
18:2	202	4	6010	14	100	1320	8	100	330	2	101
20:0	3	5	106	12	93	5	14	−80	10	18	−30
18:3	30	1	180	tr	100	160	4	98	30	1	100
Unidentified	tr	40	tr	80	—	40	23	140	45	50	78

*Mean of 4 pigs between 20 and 22 days of age.
†Trace, < 0.5 mg/day.

(Fig. 2). The dramatic increase of stearic acid and decrease of unsaturated fatty acids with 18 carbon atoms occurred in the conventional pig, but not in the germ-free pig, indicating that in the conventional pig the microflora is responsible for the pattern of fatty acids that are found in the feces.

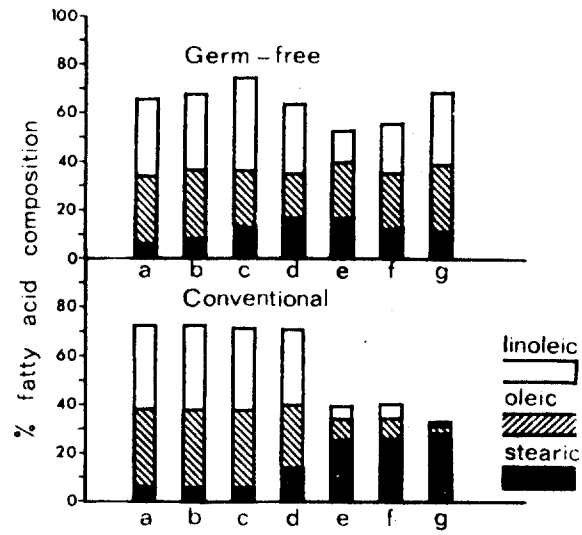

FIG. 2. The levels of stearic, oleic and linoleic acids in the feed (*a*) and ingesta of a germ-free and a conventional pig. The six regions of the digestive tract are: stomach (*b*), first half of small intestine (*c*), second half of small intestine (*d*), cecum (*e*), colon (*f*) and rectum (*g*).

The large discrepancies in values for fat digestibility by the young pig indicate that there are many unknown or uncontrolled factors that affect digestibility determinations. Before meaningful digestibility values can be tabulated, more must be known about these factors so that more control can be used in these determinations. One of the factors that should be given more consideration is the effect the microflora of the lower digestive system has on the fatty acid composition of ingesta once it has passed the regions of fat absorption and before samples are collected for digestibility determinations. This study has shown the marked effect of the microflora on the saturation of fatty acids with 18 carbon atoms, and has suggested that samples taken from the end of the small intestine rather than from the feces would give a truer indication of the pig's ability to digest fat.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. O. P. Miniats, Department of Clinical Studies, Ontario Veterinary College for the supply of the digestive tract of a germ-free pig, to Canada Packers Ltd., Toronto for the gift of the lard and tallow, and to the Ontario Department of Agriculture and Food and the Canada Department of Agriculture for financial support.

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SWINE ATHEROSCLEROSIS: THE RELATIONSHIP OF LIPIDS AND BLOOD COAGULATION TO ITS DEVELOPMENT*

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The study of the pathogenesis of atherosclerosis and its complications in man is extremely difficult. This is due in part to the fact that (1) the vascular changes developed for three to four decades prior to the clinical complications, thus making it hard to assess the amount of disease in younger subjects, (2) man is a difficult subject to control for studies of the effect of diet or other environmental factors, and (3) his vessels cannot be examined at suitable intervals during his life. Thus most human studies have to be epidemiological and indirect rather than direct experiments. This has made it imperative that suitable animals be found to facilitate studies of the pathogenesis of the disease.

Mammals,[1] birds[2] and reptiles[3] have been found to develop vascular disease, some of which is similar to that found in man. The rabbit was the first experimental animal used.[4] Although the theory has been much criticised, it appears that this species can develop a form of atherosclerosis similar to that in man if subjected to a suitable diet and period of feeding.[5] Birds have been found to develop atherosclerosis regardless of diet, but the disease can be modified by dietary means.[2] Rats[6] and dogs[7] have been used for the experimental production of the disease, but the animals used thus far have generally been considered resistant unless very modified diets are used or special drugs given, such as thiouracil to make the animals hypothyroid. The Rhesus monkey[8] and the baboon[9] have been found to develop atherosclerosis. In the case of two monkeys placed on diets rich in dairy fats and eggs for five years, one died from a myocardial infarction due to thrombosis in a coronary vessel and the other developed gangrene following a femoral artery thrombosis.[10] Atherosclerosis and its complications is not therefore a disease unique to man,

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and a variety of animals can be used for study. In order to have conditions similar to those found in man it would be best to use animals with common features. The pig is an animal with some characteristics similar to those found in man. This animal is an omnivore, has vessels similar in size, a similar coronary artery system, and susceptible animals develop a form of spontaneous atherosclerosis.[1, 11-13] The distribution of atherosclerosis is most marked initially in the thoracic aorta of these animals, but as they age the changes—particularly the fibrous plaques—become more pronounced in the abdominal, aortic arch regions. In the coronary arteries it is the proximal 5 cm. that are most heavily involved. This is very similar to the pattern reported to develop in man.[14, 15] There is intimal thickening with little fat and much mucopolysaccharide (FIGURE 1), areas of intimal thickening rich in lipids, fibrous plaques poor in lipids (FIGURE 2), and fibrous plaques with lipids deep in the base (FIGURE 3). No advanced lesions with ulceration and extensive thrombosis have yet been seen. However, most pigs in our experiments are killed when the amount of vessel disease is at about the stage shown by young men. It remains to be seen how much and what type of disease will be present in older pigs, particularly those kept on atherogenic diets. The pig, therefore, would seem to be a suitable animal in which to study the development of atherosclerosis, particularly the early stages.

We consider that there are four main factors in the development of atherosclerosis: (1) the blood constituents; (2) the hydraulics of blood flow; (3) the vessel wall; and (4) the endothelium.[16] The pig, because of its size, is a suitable animal in which these factors can be studied simultaneously. Therefore, we have attempted to examine the effect that each of these factors has on the development of swine atherosclerosis and how they can be modified by the environment.

Blood Constituents: Lipids

There is much evidence that the addition of certain fats to the diet in a variety of animals causes an increase in the amount of atherosclerosis.[1, 2, 17] There is evidence that dietary fats may be important in the development of the disease in man.[17, 18] Since the pig develops atherosclerosis while on a low-fat diet,[1] it was of some interest to observe the effect of feeding various dietary fats to swine.

Three experiments have now been completed. In the first experiment,[19] a comparison was made between the effect of margarine and the effect of butter. Thirty-three swine matched for age, sex, weight, and breed were divided into three groups of 11. The pigs in Group A received the basic hog ration and butter to forty per cent of their calories; in Group B the

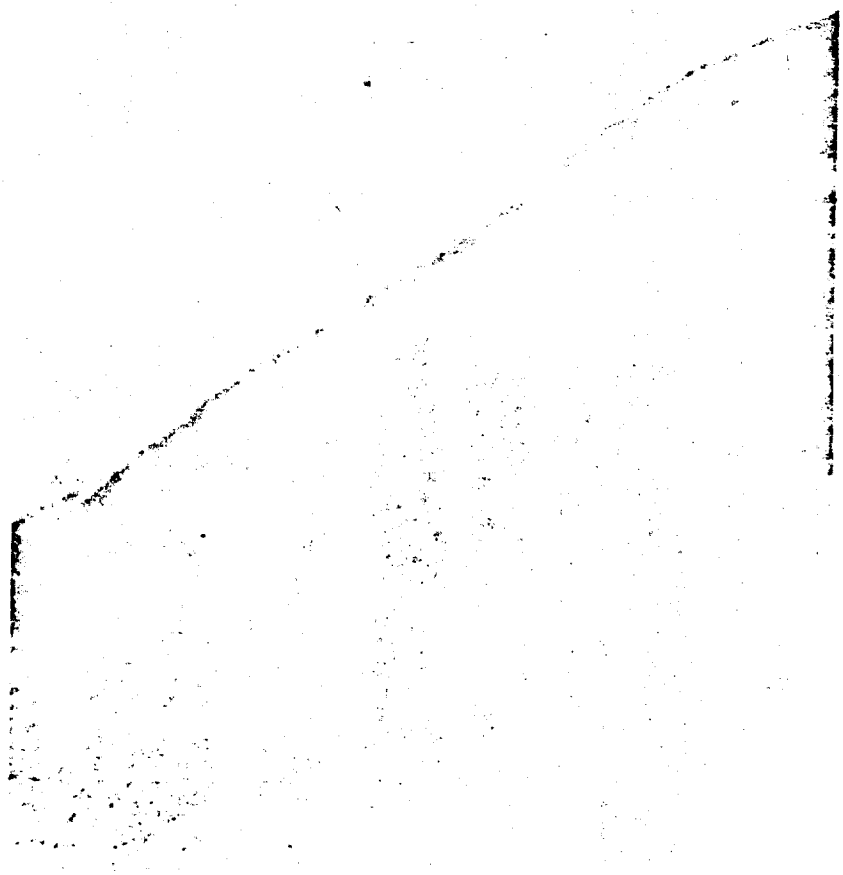


FIGURE 1. Shows an atherosclerotic plaque from a pig fed egg yolk. The thickened intima has an accumulation of sudanophilic material on its surface. The tissue was stained with Oil Red O and counterstained with Light Green and Harris Hematoxylin. × 100.

basic hog ration and margarine to forty per cent of their calories; and the pigs in Group C, the basic hog ration only. The animals were killed over a period of 3 to 9 months. The mean serum cholesterol level at the end of the experiment in the animals fed butter was not significantly diff-

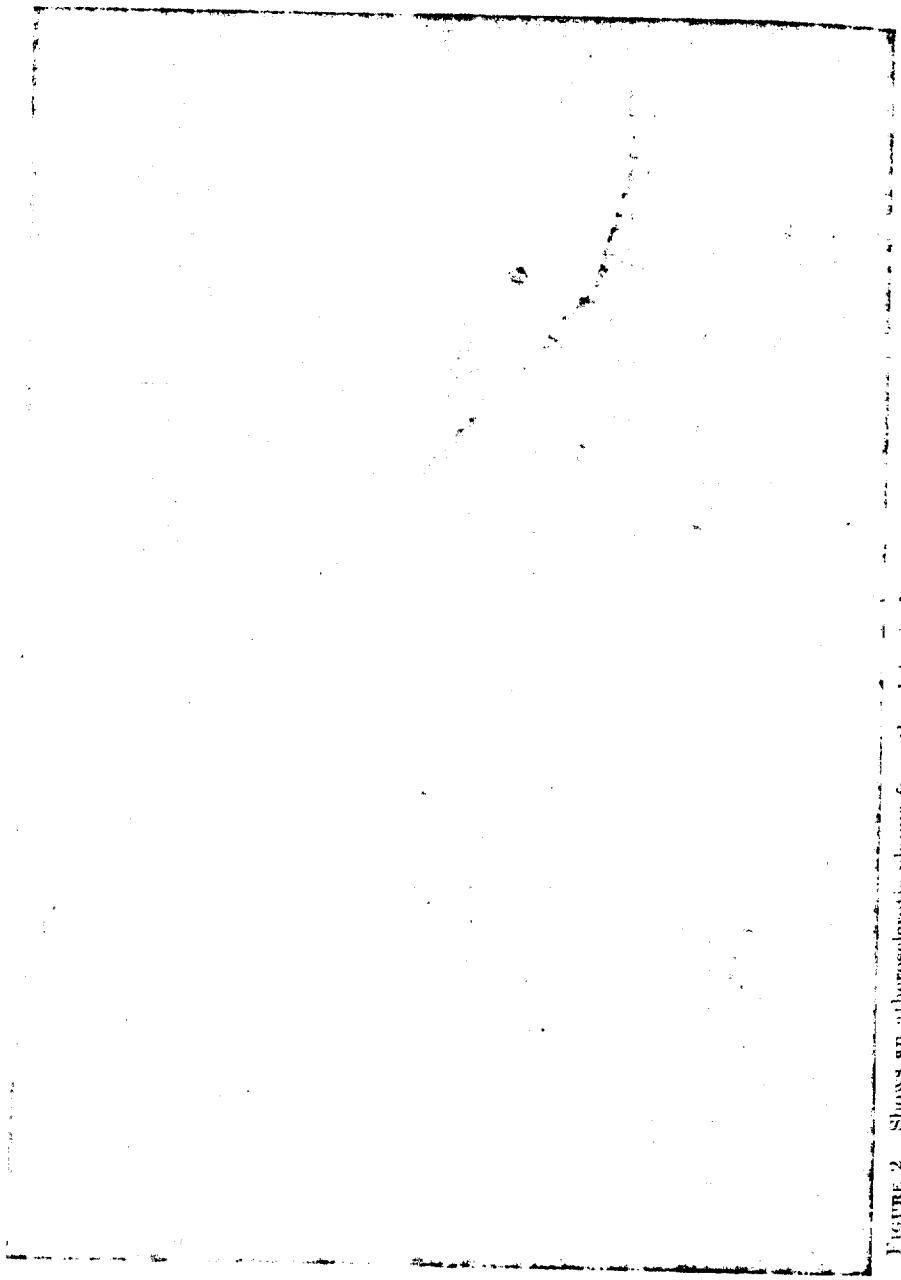


FIGURE 2. Shows an atherosclerotic plaque from the abdominal aorta of a pig fed egg yolk stained with Oil Red O and counterstained with Light Green and Harris Hematoxylin. This is a film as plaque that [illegible]

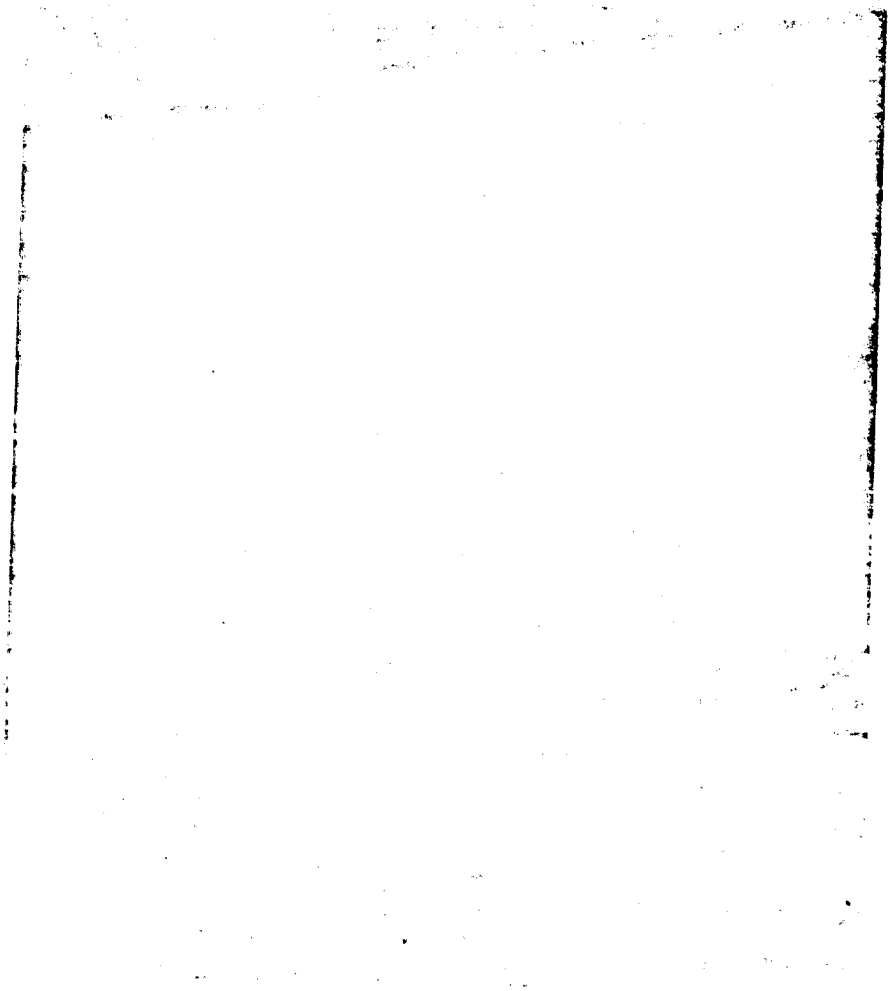


FIGURE 3. Shows an atherosclerotic plaque from a pig fed egg-yolk, stained with Oil Red O and counterstained with Light Green and Harris Hematoxylin. Fat staining material can be seen deep in a fibrous plaque. No fat was present on the surface. ×240.

ferent from that for the animals fed margarine or the low-fat diet. However, the animals fed butter had the greatest amount of aortic atherosclerosis, which was about three times that for the margarine group and about three and one-half that for the control group. There was little difference between the margarine and control groups. This experiment

showed that in the pig it is not the quantity of fat in the diet that is important, but the type of fat. Furthermore, the increased atherosclerosis seen with the butter-rich diet was not associated with an increase in the serum cholesterol or phospholipid. It would appear from the evidence in this and other studies[1-20] that whatever the role of blood lipid is in the development of atherosclerosis, its elevation is not necessary for development of the disease.

In the second experiment a comparison was made between the effects of egg-yolk and butter.[1] A group of 33 pigs was divided into 3 groups of 11. The pigs in Group A were given the basic hog ration plus 33 per cent of their calories as butter; in Group B, the basic ration plus 33 per cent of their calories as egg-yolk; and those in Group C were maintained on the basic low-fat ration. The animals were maintained on the diet for from 4 to 12 months. The animals given butter showed little change in their serum cholesterol levels from that of the controls, whereas the pigs given the egg-yolk showed a progressive increase in their serum cholesterol and phospholipid that was significantly different from the values for the butter-fed and control animals. The animals fed egg-yolk showed at 40 weeks a significantly greater mean concentration of the standard Sf 0-12, 12-20, and 20-100 lipoprotein fractions. The animals given butterfat showed a significant increase in the standard Sf 12-20 fraction when compared with the controls, but were otherwise similar. The mean amount of the aorta involved with atherosclerosis in the egg-fed group was 6 times that in the controls, whereas in the butter-fed pigs it was 3 times that found in the controls. A positive correlation was found for the relationship between the blood cholesterol level and the percentage of aorta involved with atherosclerosis in the egg-yolk group. However, in the butter-fed pigs the correlation was the opposite and in the controls there was no significant correlation. This evidence shows that the effect of an isocaloric amount of egg yolk on the development of swine atherosclerosis is considerably greater than that of an isocaloric amount of butter. In the egg-fed swine the increased atherosclerosis was associated with elevation of the serum cholesterol and increase of low-density lipoprotein fractions. Thus, although increase of the serum cholesterol or lipoprotein levels are not essential for the development or acceleration of swine atherosclerosis, when they are increased the amount of disease that appears is much greater.

In the third experiment[21] 32 swine were divided into 4 groups of 8. Group A was given the basic hog ration with 25 per cent of their calories as lard; Group B was given the basic hog ration and 25 per cent of their calories as lard plus an amount of cholesterol equal to that found in the egg-yolk diet given to Group C. Group C was given the basic ration plus

25 per cent of their calories as egg-yolk. Group D was given the basic dog ration only. The animals given the diet containing lard and cholesterol had the greatest mean serum cholesterol level (TABLE 1) and the highest mean standard Sf 0–12, 12–20, and 20–100 beta lipoprotein fractions (TABLE 2). The animals given lard had values that were only slightly higher than those for the controls. The animals given egg-yolk had serum lipid and lipoprotein values that were between those for the animals given lard and those given lard plus cholesterol. All animals given added fat showed a significantly greater involvement of the aorta with atherosclerosis than did the controls (TABLE 3). The greatest involvement in terms of total area involved was found in the animals given lard and cholesterol. Fibrous plaque development was similar in the lard cholesterol and egg-yolk group. Both these groups had significantly more fibrous plaque development than that seen in the control group (FIGURE 1). This study showed that dietary fat not only influenced development of fatty streaks but also the later development of fibrous plaques. There has been some question as to whether diet in man can influence the development of fatty streaks and fibrous plaques.[22] The results of this study showed

TABLE 1
TERMINAL MEAN SERUM CHOLESTEROL AND PHOSPHOLIPID VALUES
(MG. PER CENT)

Group	Cholesterol	Phospholipid
Control (C)	114.5	109.0
Lard (L)	134.8	143.25
Egg yolk (EY)	158.25	145.6
Lard cholesterol (LC)	236.1	188.5
Significance of differences between means		
C *vs.* L	$p < .4$	$p < .1$
C *vs.* EY	$p < .1$	$p < .02$
C *vs.* LC	$p < .001$	$p < .02$

TABLE 2
TERMINAL MEAN STANDARD SF LIPOPROTEIN VALUES (MG. PER CENT)

Lipoprotein fraction	Sf 0–12	Sf 12–20	Sf 20–100	Sf 100–400
Control	127.6	5.1	3.4	0.6
Lard	139.8	4.4	2.2	1.6
Egg yolk	194.5	24.9	9.9	2.6
Lard cholesterol	297.8	103.4	29.2	5.2

TABLE 3
MEAN AREA INVOLVED WITH ATHEROSCLEROSIS (PER CENT OF AORTA)

Group	Total	Fibrosis
Control (C)	16.14	1.387
Lard (L)	21.83	3.041
Egg yolk (EY)	32.78	4.229
Lard cholesterol (LC)	41.95	4.137
Significance of difference between means		
C vs. L	$p < 0.3$	$p < 0.03$
C vs. EY	$p < 0.01$	$p < 0.01$
C vs. LC	$p < 0.01$	$p < 0.01$

that it can in swine, but that this is related not to the quantity of fat but to the type of fat and that it is not directly related to the serum cholesterol level in the blood. It would therefore appear that in human studies greater attention must be paid to the exact type of fat consumed.

Another point of interest is the evidence from this study that elevation of serum cholesterol in swine is related to the triglyceride and cholesterol content of the diet. This is similar to the effect reported in man by

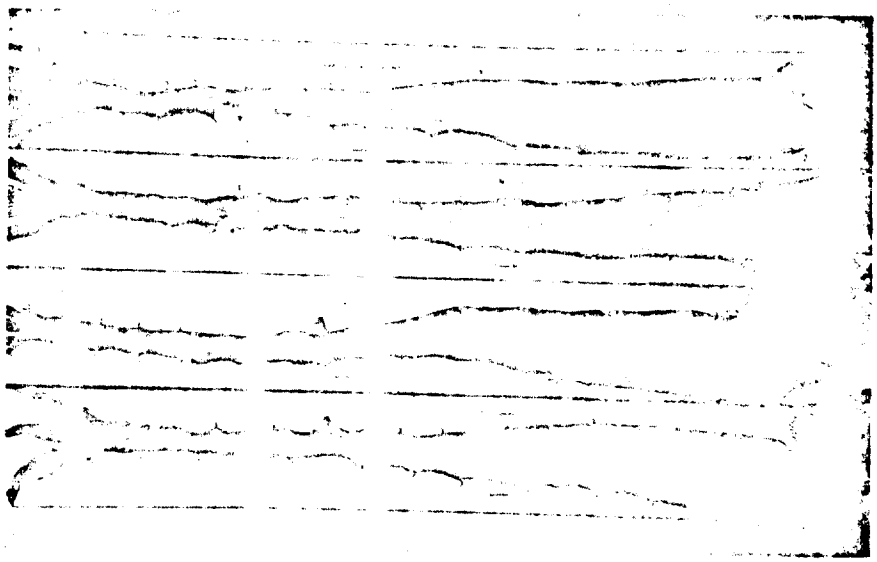


FIGURE 1. Four aorta of swine can be seen. The animals were fed from the top down: lard and cholesterol, egg yolk, lard, and a low fat diet. The aortas are stained with Herxheimer's Sudan IV. The dark areas show a typical distribution of fat staining material after nine months of feeding.

veridge[23] and confirmed by others.[24, 25] It seems likely therefore that serum cholesterol and beta lipoprotein levels in swine depend to some extent on these two dietary components.

When swine are given diets rich in certain triglycerides and cholesterol some develop higher serum cholesterol levels than others. The problem in the human with hypercholesterolemia has been in part whether this disorder is due to an inborn metabolic error with excess production of cholesterol[26] or the result of susceptible subjects receiving hypercholesterolemic diets.[27] Since humans living in parts of the world where diets are low in fat have serum cholesterol values similar to those found in corn-fed pigs,[28] it seems likely that much of the hypercholesterolemia seen in western man could be due to dietary factors. It can be seen that when swine are given foods that are hypercholesterolemic in man, they develop a distribution of serum lipid values similar to those seen in North American man. It would thus appear that hypercholesterolemia may be largely due to dietary factors, and we must reconsider what is meant by man's normal serum cholesterol level. As has been suggested, man's normal level may be less than 150 mg. per cent.

The changes in vessel pathology, particularly the coronary arteries, associated with various diets are of some interest. The aortic changes appeared in the aortic valve area, orifices of the vessels of the head and neck, about intercostal vessel orifices, the abdominal aorta, and the trifurcation of the aorta. The earliest changes were fatty streaks or intimal thickening, which in some areas progressed to fibrous plaques. Fibrous plaque development was most marked in the abdominal aorta with some in the aortic arch. In time the most advanced lesions were predominantly in the abdominal aorta. As the disease progressed the scattered floccular lesions tend to become confluent. As this occurred changes became readily detectable in the iliac, renal, and carotid arteries.

Coronary artery disease became prominent after the disease in the aorta had become extensive. We have only seen slight intimal thickening of the coronary arteries in our control animals, whereas the animals fed egg-yolk or lard plus cholesterol have shown considerable involvement and after seven months on these diets, all animals showed lesions. Of the various diets used, it was those containing egg-yolk or lard plus cholesterol that produced the most coronary artery disease. It would appear that—as in man—coronary artery atherosclerosis tends to become grossly detectable after the aortic involvement has become extensive.[14] However, it does not appear to be a different disease from that seen in the aorta. The disease is most marked about the coronary artery orifices and the proximal 5 cm. of the vessels (FIGURE 5). In some cases the degree of involvement after one year in swine given lard plus cholesterol or egg-yolk reduced the lumen

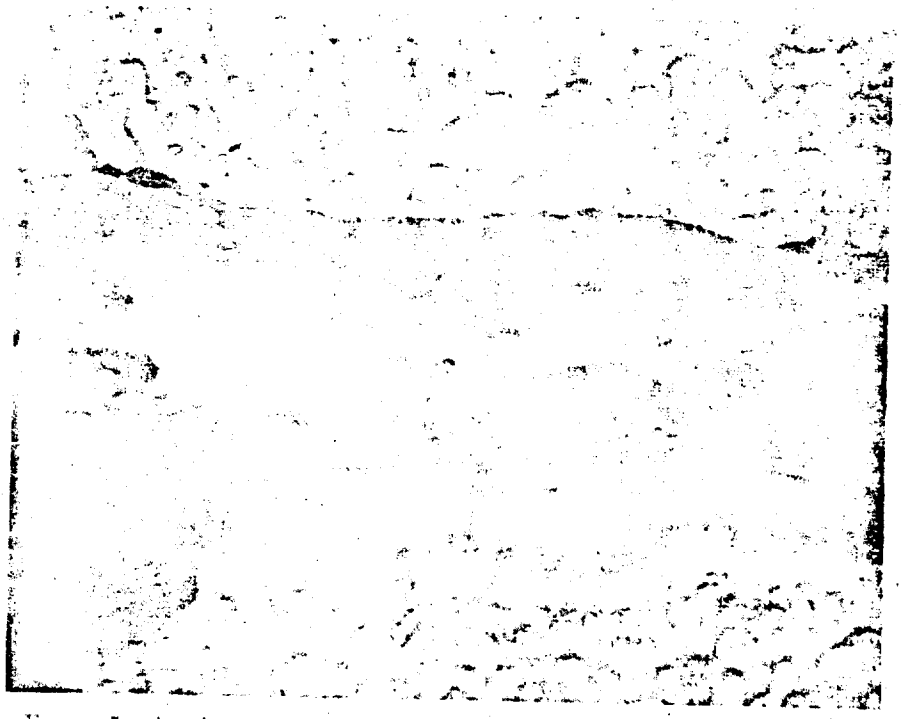


FIGURE 5. A swine coronary artery is shown stained with Herxheimer's Sudan IV. The aortic orifice is at the left. Fat deposits are prominent as the darkly stained areas and are seen about vessel orifices showing a typical butterfly-like pattern with deposits lateral to the openings and sparing the proximal lip. (The cardboard used as a background shows a pebbled surface.)

size by 25 per cent. It seems likely that with a longer period of time and with various diets these changes would become more extensive.

Hydraulics of Blood Flow: Hydraulic Forces and the Topography and Pattern of Development of Atherosclerosis

In all animals that develop atherosclerosis it has been noted that there is a remarkably constant pattern of development for each species.[29, 30, 31, 32] In the pig the disease starts in the region of the aortic valve ring, lesser curvature of the aorta, base of the great vessels of the head and neck, about intercostal vessel orifices, and the trifurcation of the aorta. About the intercostal vessels, the disease begins lateral to the orifice and gradually extends in a butterfly pattern. With further extension the lesions at

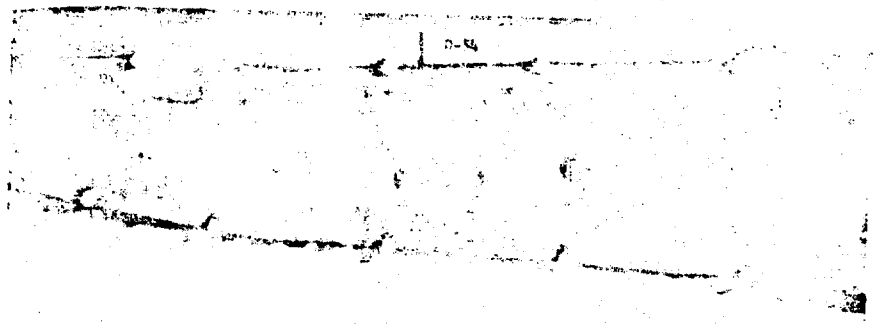


FIGURE 6. The thoracic aorta of a swine has been opened longitudinally and stained with Herxheimer's Sudan IV. The areas containing fat are seen in the region of the vessel orifices producing a typical butterfly-like pattern. The deposits are heaviest lateral to the orifices, and the proximal lip is spared. There is a tendency for the lateral deposits to proceed down the aorta producing a ladder like pattern as the areas become confluent. Flow was from the right to the left.

multiple orifices become confluent, producing a ladderlike pattern (FIGURE 6). In the case of the bifurcation or trifurcation of the aorta the initial disease occurs at the lips of the trifurcation and extends down the lateral side of the iliac vessels (FIGURE 7). Most of the lesions show a linear pattern related to direction of blood flow. Frequently these linear streaks have a barred or ribbed pattern similar to the ripple marks on a wave-

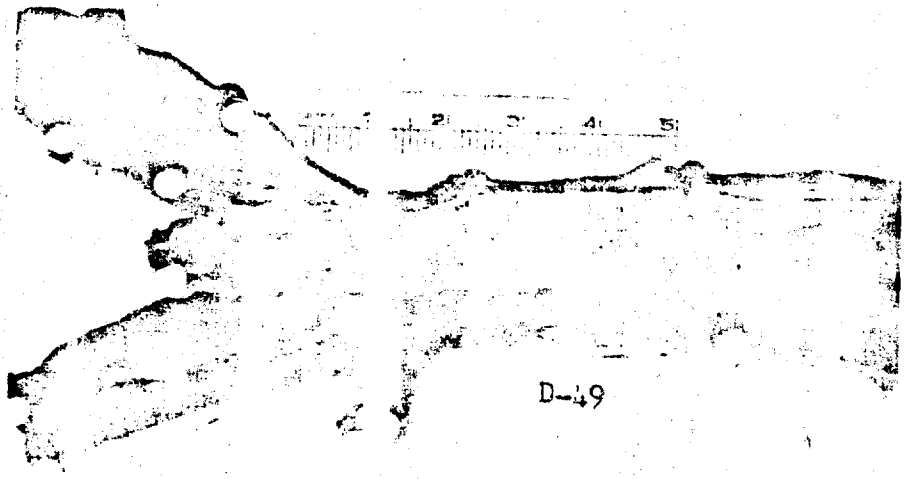


FIGURE 7. A swine aorta is shown opened in the region of the iliac vessels and stained with Herxheimer's Sudan IV. The atherosclerotic lesions are seen at the lips of the trifurcation and extend down the lateral aspects of the distributory vessels. The linear streaks in the abdominal aorta [illegible]

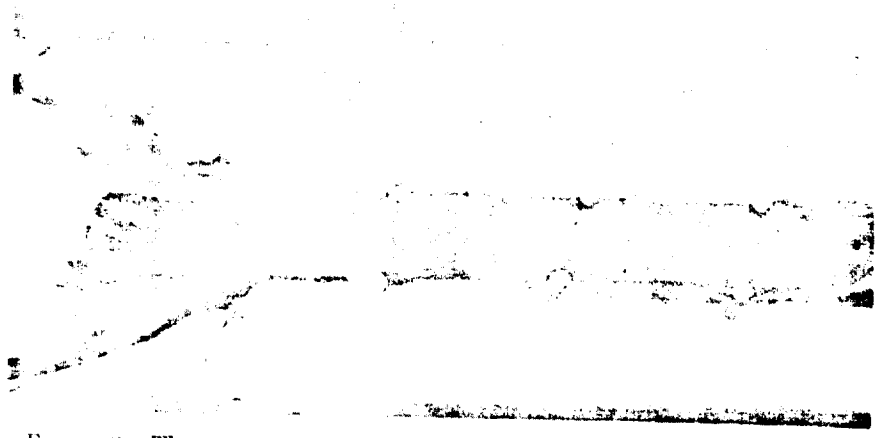


FIGURE 8. The aorta of a swine is shown opened in the lower abdominal and iliac region and stained with Herxheimer's Sudan IV. A dark linear streak in the aorta shows a barred or ribbed pattern.

washed beach (FIGURE 8). It has been suggested that these are sites of greater mechanical strain and vessel injury.[33] Injury to vessels is known to localize and promote the development of atherosclerosis.[34] Another suggestion has been that the hemodynamic factors associated with blood flow are important.[16, 29] It has been suggested that injury may change vessel wall permeability, leading to increased accumulation of lipids.[35] A further mechanism could be the action of the hemodynamic forces on the particulate matter in circulating blood. At points where there is turbulence and eddy formation, the elements of blood may be thrown against the vessel surface. We have attempted to explore this latter possibility.[16, 29]

A group of plastic or brass models of various vessel configurations were made. These were coupled into extracorporeal circulations connected to the carotid artery and jugular vein of anaesthetized swine. The blood was allowed to flow through the shunts for a period of 20 minutes with a rate of flow that was between 200 and 700 ml. per min. All the experiments were conducted at room temperature. In the flow chamber models of the vessel bifurcation the deposit that formed was located at the site of the bifurcation. The proximal limb tended to be clear. The deposit extended across the hip of the bifurcation and down the lateral aspects of the branched vessels (FIGURE 9). This is similar to the pattern of development of atherosclerosis at the aortic bifurcation in man and the aortic trifurcation in swine.

Cinephotography of the blood flow pattern at this site in the flow chamber

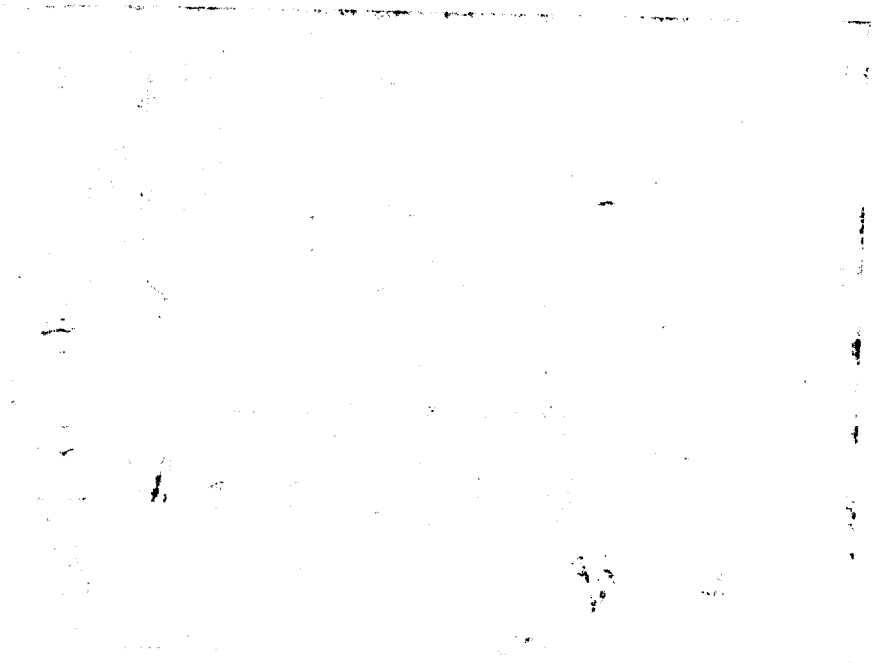


FIGURE 9. A plastic flow chamber is shown with a typical advanced deposit formed in the area of the bifurcation. The deposit formed on the lips of the bifurcation and extended down the lateral wall of the distributing vessels. Flow was from the left to the right.

model showed that there is a pocket at each side into which the blood swirls, setting up eddy currents. It is probable that this turbulence and eddy formation is to a large extent responsible for the deposits occuring at these sites.

In a model with a series of right angled branches the deposits occur at the lateral aspects of the orifices. The area proximal to each orifice was to be spared, whereas the lateral aspect showed the heaviest involvement. In time these lateral extensions at each orifice became confluent producing a ladderlike pattern similar to atherosclerosis about intercostal vessel orifices. In the flow chamber the punctate character of the early deposits gave an indication of the forces involved. The curved lines of the deposit extending into the sides of the orifice suggest that these appear with the swirling of blood from the lateral regions. The deposits become more intense in the distal parts of the chamber where there is a reduced volume of flow. The area of deposit in the distal parts may have some bearing

on the tendency for atherosclerosis to be predominant in the abdominal aorta (FIGURE 10).

Heavy deposits occur along the lesser curvature of tubing in flow systems. This is also true of atherosclerosis in the arch of the aorta and in the coronary artery.

The area of deposit in the flow chamber frequently had a floccular character that is seen in some of the early stages of atherosclerosis in men and animals. These individual deposits become confluent, forming linear streaks that can have a barred or ribbed pattern. This pattern, which is similar to the ripple marks of a wave-washed beach, is also seen with the flow chamber deposit. It seems that this particular pattern may be related to the pulsatile nature of blood flow in a high flow, pulsatile system.

All this evidence suggests that the influence of the hydraulic forces on the particulate matter of circulating blood could explain a large part of the early stages of atherosclerosis. However, in order to explore this relationship, it is necessary to know the nature and sequence of events in the formation of the deposits.

In the flow chamber the first deposit is primarily composed of the blood platelet with considerable numbers of white cells, red cells, and fibrin appearing later. To decide whether this mechanism is of importance it is necessary to observe whether the platelet deposits on the endothelium in normal intact animals and whether they are incorporated into the intima, producing the subsequent changes seen in the early stages of atherosclerosis.

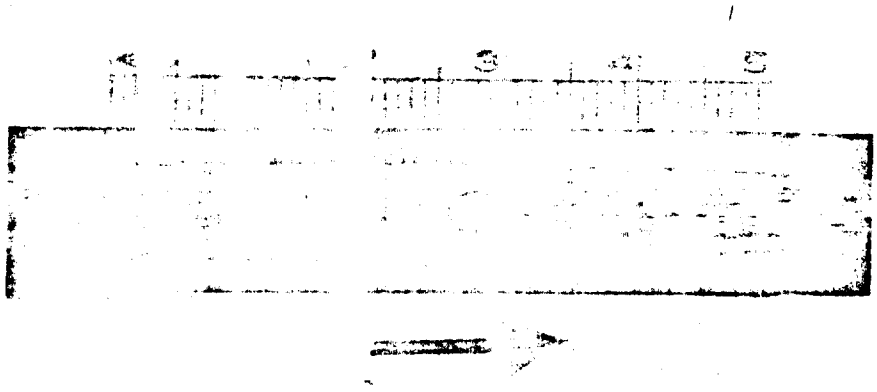


FIGURE 10. A typical pattern of deposit formation is seen in an opened brass flow chamber. The narrow channel in the center shows two orifices of the right-angled distributing branches. The arrow indicates the direction of blood flow. The dark deposits are heaviest lateral to the orifices, the proximal lip being spared. Close examination of the distal opening shows typical streaming with lines of deposit directed towards the orifice. Increased deposits are evident in the lower limits of the channel where the flow was reduced.

Endothelium and Vessel Wall

To study this problem endothelial membranes were prepared from swine of all ages.[16, 36] These membranes, which are prepared essentially by the method described by Poole, *et al.*,[37] consists of the endothelium spread as a sheet, on a glass slide. Examination of swine endothelium has shown that deposits of cellular material are frequently seen on the surface. These deposits are composed of some red and white cells and a large amorphous mass which resembles clumped platelets (FIGURE 11). By the use of radioisotope techniques we consider that the bulk of this amorphous material is the blood platelet.

The microthrombi were found to have a topography and pattern similar to what might be expected from the flow chamber experiments. About intercostal vessel orifices the deposits are lateral to the opening with sparing of the proximal lip (FIGURE 12). In some cases the curved pattern of the deposits seen sweeping toward orifices in the flow chamber experiment can also be seen on the endothelium. About the trifurcation of the aorta deposits are found extending down the lateral sides of the distributing vessels. This was also the pattern of deposits in analogous flow chamber

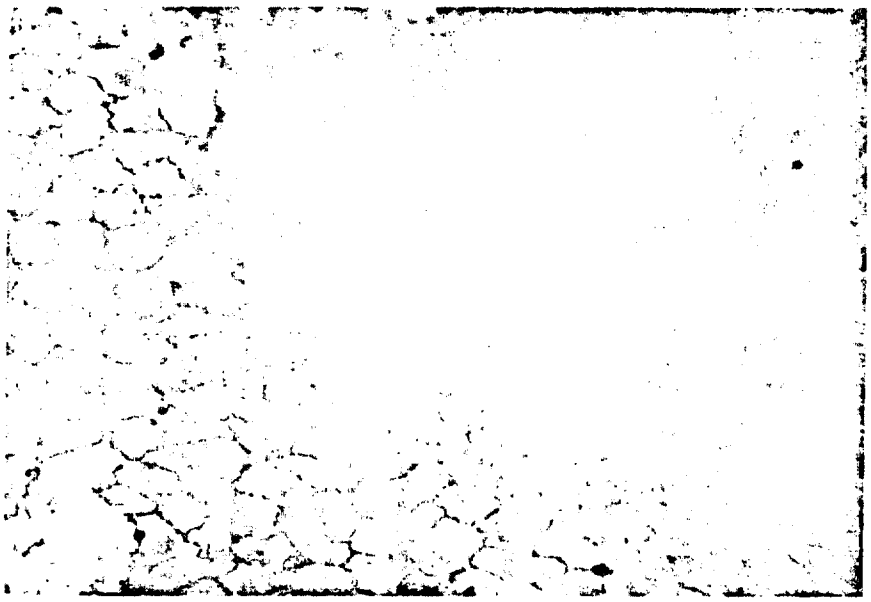


FIGURE 11. An endothelial preparation is shown stained with silver nitrate and Harris Hematoxylin. A mixed deposit of cells is seen on its surface. The granular appearance of the great mass of the deposit is believed to be due to adherent platelets. The darker bodies are mixed red and white blood cells. ×400.

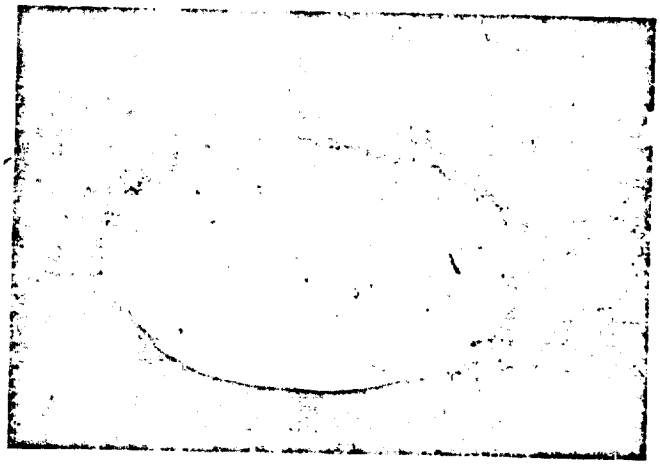


FIGURE 12. An endothelial preparation about a vessel orifice is shown stained with silver nitrate and Harris Hematoxylin. Microthrombi can be seen as the dark areas arching away from the lateral orifice regions. The proximal lip is spared. Blood flow was from the top down. 13.

models. The deposits are frequently in linear streaks with a floccular or ribbed pattern. These findings show that microthrombi occur on endothelium of normal intact swine and that the deposits have the same pattern as early atherosclerosis.

The question of whether these deposits are involved in the development of intimal thickening and atherosclerosis rests on determining whether they persist and are incorporated into the intima. Serial cross-sections made through regions where thrombi occur has shown that this does take place (FIGURE 13). Some thrombi can be found with a layer of endothelium above and below the deposit, indicating that they are being incorporated into the intima. Similar findings have been reported from studies in man.[38,39] Chandler and associates[40] have recently demonstrated that monocytes can phagocytose platelets giving rise to foam cells. More recent work from their laboratory[41] has shown that the injection of platelet thrombi into rabbits can lead to changes in the pulmonary arteries similar to the intimal changes seen in rabbit atherosclerosis. In their experiments it was of interest to note that within four days the pulmonary emboli were completely covered with endothelium and that within five days monocytes had started to become transformed into foam cells by ingestion of platelet material. This evidence suggests that when microthrombi occur on intact endothelium they can be incorporated into the vessel walls more rapidly than when microthrombi occur on areas denuded of endothelium. There is considerable experimental evidence that fibrin clots can give rise to fibrous atherosclerotic lesions.[42] There now seems no doubt that deposits of particulate matter from the blood

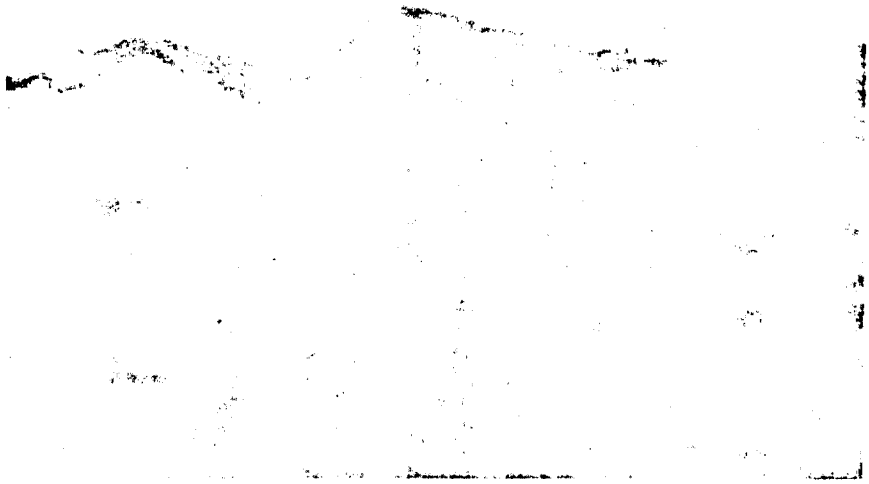


FIGURE 13. Shows a microthrombus on the endothelium. The beginning of incorporation by the endothelium can be seen at the righthand edge of the mass. (Stained with silver nitrate and Harris Hematoxylin.) ×576.

can give rise to most of the known features of atherosclerosis. It is known that factors which damage the endothelium cause the formation of microthrombi. Damage to the endothelium can be due to direct injury or injury to other layers of the vessel wall. Adrenaline is one factor that has been reported to damage all layers and cause adherence of platelets and white cells to the endothelium.[45] It is clear that attention must be paid to the function of the vessel wall when studying the relationship of microthrombi to the development of atherosclerosis.

There is considerable experimental evidence that vessel wall injury both localizes and promotes the development of atherosclerosis.[34]

Thrombi can become vascularized,[44] and, when this occurs in thrombi which are incorporated into the intima, this may become a factor in the development of intimal hemorrhage. There is some experimental evidence that suggests that this occurs, and numerous studies in man have indicated the role of intimal hemorrhage on the later stages of atherosclerosis and its complications.[45] Intimal hemorrhage has not been found to be of any importance in the stages of atherosclerosis so far examined in swine.

Blood Constituents: Blood Coagulation

There is considerable evidence that dietary fats and serum lipids are related to the development of atherosclerosis and its complications.[17]

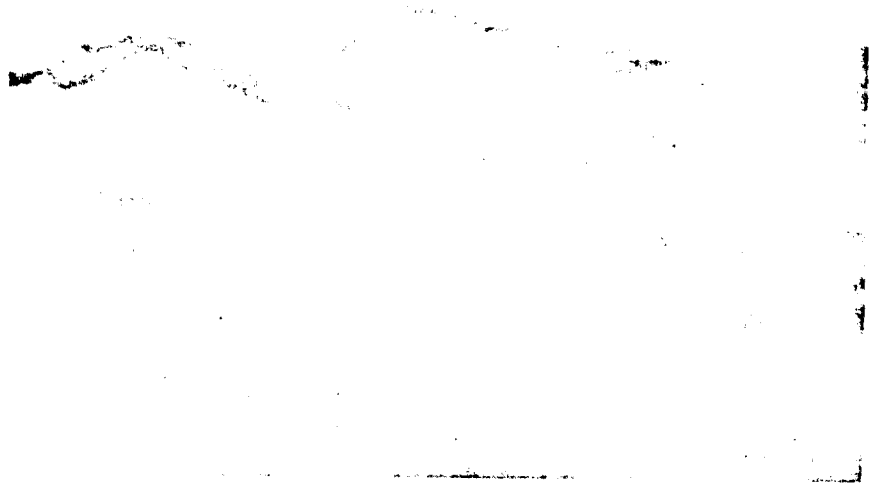
The composition of the deposits may be influenced by the blood lipid level. We have found a significant, positive correlation between the serum cholesterol level in man and the cholesterol level of once washed platelets.[29, 46] Although this evidence does not indicate whether the lipid is a part of the platelet or plasma trapped on its surface, it does show that the lipid level of platelets is correlated with that of the plasma. Thus one might expect a higher lipid content in a platelet deposit formed from lipid-rich plasma.

In swine with various lipid levels we have examined the lipid content of thrombi formed in extracorporeal shunts. These studies showed that the animals with the higher serum lipid levels had the more lipid-rich deposits.[47] This may be of some importance, since it has been shown that lipids can excite a tissue reaction. It is possible therefore that lipid-rich deposits cause a more marked intimal reaction.

The influence of lipids on the stability of thrombi has been studied with respect to the fibrinolytic mechanism.[48-50] Several investigators have shown that some lipids can inhibit this mechanism. In particular the studies have incriminated saturated fats, particularly dairy fats and egg-yolk. It is known that experimental thrombi formed in animals with hyperlipemia are resistant to lysis.[48] The structural stability of thrombi composed primarily of platelets is probably due to the fibrin component. It is conceivable then that the action of the fibrinolytic mechanism can influence the stability of platelet thrombi.

Another mechanism whereby lipids can influence the extent of thrombi formed is by enhancing deposition. This can be done through two mechanisms, (1) damage to the endothelium and (2) enhancement of blood coagulation. The evidence for the first point is incomplete. It is known that lipids can permeate the endothelium, and Shimamoto[51] has claimed that when this occurs the endothelium is altered, enhancing platelet and white-cell deposition.

The evidence for the influence of blood coagulation on thrombus formation is more direct. There is considerable data that anticoagulants can inhibit experimental thrombus formation.[52-54] We have studied this problem using the extracorporeal shunts adapted as a quantitative method for studying thrombus formation in a high flow, pulsatile system. In principle, since flow and surface are constant, we can study the effect of changing the character of circulating blood in the shunts. These studies have shown the following: (1) That dicumarol in low doses enhances thrombus formation, whereas in high doses there is some inhibition[34, 55] and (2) immediately after the administration of large doses of heparin, thrombus formation is completely inhibited but when the clotting time returns to the baseline thrombus formation is enhanced.[56]



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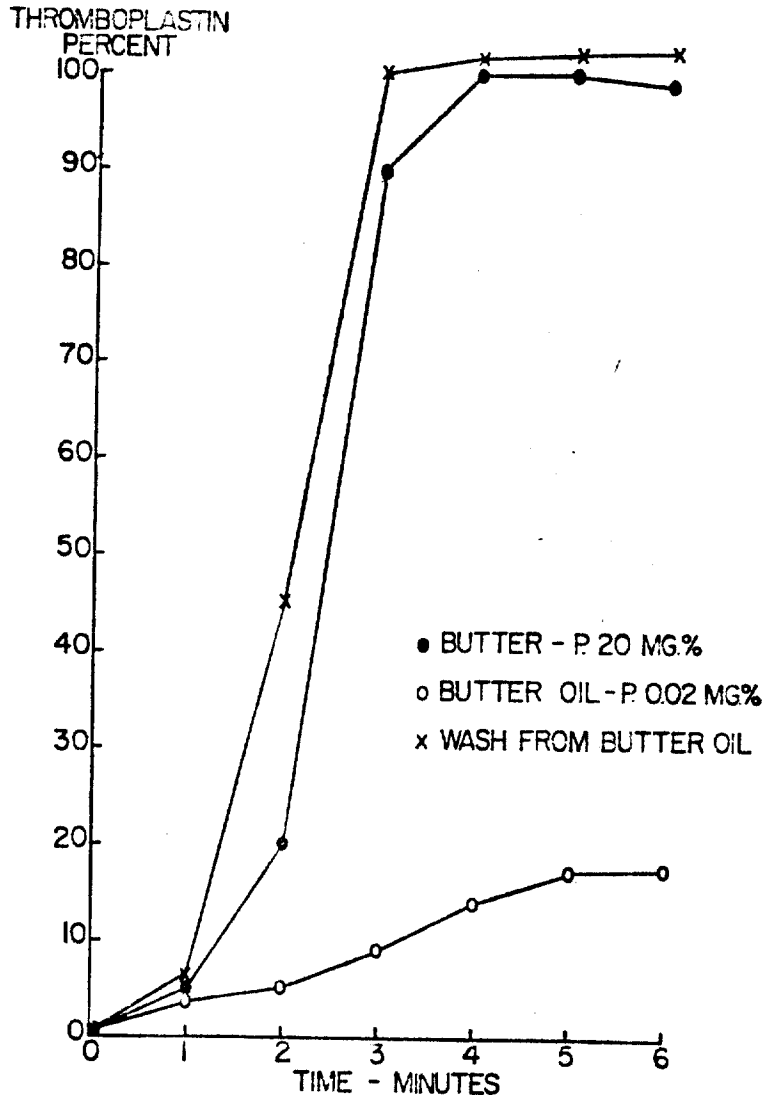

FIGURE 14. The effect of Butter and Butter fractions on Thromboplastin Generation. Normal $Al(OH)_3$-treated plasma and normal serum were used in the test system. Butter ●—●, Butter Oil, ○—○ and the wash from the butter ×— ×, were used in place of platelets. These materials were suspended in Imidazole Buffer, *p*H 7.3, prior to testing. The Butter Oil was made by washing the butter. The Phospholipid (P) content of the butter was 20 mg. per cent (as lipid phosphorus); whereas that of the washed butter (Butter Oil) was 0.02 mg. per cent (as lipid phosphorus). The major portion of the phosphorus was transferred to the butter during the washing procedure. If no platelets are used in the test system, the curve does not reach more than 6 to 12 per cent Thromboplastin at the 6th minute.

formation of thrombi in an *in vitro* system. It remains to be seen whether their action will be in an *in vivo* system.

Summary

Swine develop atherosclerosis which is similar in morphology and topography to atherosclerosis in man. The extent of the disease is related in part to the type of diet and time. Diets rich in dairy fat, egg-yolk, butter, or lard accelerate the development of the disease. These dietary factors enhance blood coagulation and thrombus formation and some raise the serum lipid level. In addition to the study of these blood constituents on the development of atherosclerosis, we have examined the role of the endothelium and hydraulic forces of blood flow. These studies have shown that the hydraulic forces can localize and promote the formation of deposits on the endothelium. Deposits incorporated into the intima by overgrowth of the endothelium can give rise to atherosclerosis; although these changes occur to some extent in normal animals on low fat diets, the changes are accelerated in animals given diets rich in certain fats. These dietary factors can influence the mechanisms described by their effect on serum lipids, blood coagulation, thrombosis, and the endothelium. The possible relationship of all these factors has been examined in the pig.

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Analyses of Pecan, Peanut, and Other Oils by Gas-Liquid Chromatography and Ultra-Violet Spectrophotometry

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Comparative analyses of oils obtained from 12 varieties of pecans, 11 of peanuts, two of avocados and one each of citrus seed, Wesson oil, corn, and of lard by GLC and UV procedures showed good agreement except for Wesson oil, corn oil, and lard. Iodine values computed from GLC results checked Wijs values closely. Oleic acid ranged from 51 to 77% in pecans and from 43 to 64% in the peanut with linoleic acid in complementary percentages.

Level of palmitic acid in the peanut varied directly with level of linoleic acid, in the avocado it apparently varied directly with level of hexadecenoic acid while in the pecan the level of palmitic remained constant even though the level of unsaturated acids varied widely. Both peanuts and pecans were locally grown under similar environmental conditions.

INFORMATION on the composition of certain natural oils was needed for an experiment designed to test cholesterogenic effect of oils in diets on rat blood serum. Analyses were made on the oils by methods of gas-liquid chromatography (GLC) and by ultra-violet (UV) spectrophotometry. Analyses by the two procedures were compared on oils expressed in the laboratory from 11 varieties of peanuts, 12 of pecans, 2 of avocado, and on market purchased samples of citrus seed oil, Wesson oil, corn oil, and lard.

Apparatus and Methods

Gas-liquid chromatograms were obtained with an A-100 Aerograph using a 4-filament thermal sensitive cell with an oven temperature of 200C and a gas flow rate of 60 ml/min of helium. One to 3 lambda, the volume depending upon the oleic acid content of the sample, of a 20% solution of the fatty acid methyl-esters (Id) in benzene was placed in the heated injection chamber. Passage through a 5-ft. column packed with 10% Lac 446, a glycol adipate polymer, on 30-60 mesh firebrick gave sharply shaped curves for the methyl esters of the fatty acids with 14 to 20 carbons in their chains. The area under each curve was computed by multiplying the height of the peak by width at middle height and ascribed to each fatty acid as indicated by the time it left the column.

Iodine values were determined by the Wijs method (2), and the polyunsaturated acids by the UV spectrophotometric procedure (3).

Samples

The oil samples were chosen from 12 popular varieties of pecans grown in north-central Florida, and from 11 varieties of peanuts which have been or probably will be recommended to growers (4). The pecans were purchased from an Alachua County merchant who handled locally-grown nuts. The peanuts, which included Spanish, runner, and Virginia types, were all grown on the Florida Experiment Station farm in 1958 under the same supervision and under the same environmental conditions. The pecan and peanut oils were cold pressed in a Carver laboratory press. The 2 avocado samples, supplied by a grower in the Miami area, were dried and the oil extracted with hexane. The citrus seed oil was a commercial Florida product. The Wesson oil, corn oil, and leaf lard were purchased in local stores. The lard organoleptically showed a trace of rancidity. All fats were kept frozen under prepurified nitrogen until analyzed.

Results and Discussion

The GLC analyses are given in Table I. The analyses of oils from the 11 peanut and 12 pecan varie

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TABLE I
Oil Analysis by Gas-Liquid Chromatography

Item	Palmitic	Hexadecenoic	Stearic	Oleic	Linoleic	Linolenic	Arachidic
	%	%	%	%	%	%	%
Peanut[a]							
Spanish 18-38-47	12.9	0.2	4.5	43.1	32.5	1.5	0.7
N. Carolina 2[b]	10.5	T[c]	4.3	51.9	26.2	1.3	0.6
Va. Runner G-26	10.3	0.5	4.7	52.3	25.1	1.5	0.9
Spanette	10.9	0.4	3.7	52.5	25.7	1.3	0.7
Ga. Bunch 119-20	11.1	T	4.1	54.4	23.2	1.4	1.1
Va. 56R	9.6	0.6	4.4	54.5	23.8	1.4	0.9
Early Runner	9.7	...	3.1	55.6	24.7	1.1	1.0
Va. Bunch G2	10.1	...	3.7	55.7	23.7	1.3	0.7
Florigiant	10.3	T	4.1	55.7	22.9	1.6	0.7
Dixie Runner	9.3	...	3.1	58.5	22.4	1.1	0.7
Fla. 393-47	7.3	...	4.3	63.5	17.7	1.4	1.1
Pecan							
Moneymaker	6.3	0.2	2.6	51.0	37.8	1.7	0.4
Mobile	7.1	...	2.7	56.8	31.4	1.8	0.2
Big Z	5.9	T	2.9	59.7	30.3	1.6	0.4
Randall	6.0	T	2.9	61.3	28.2	1.5	0.1
Teche	6.1	T	3.0	63.4	25.7	1.3	0.5
Frotscher	6.5	T	2.5	63.9	25.5	1.3	0.2
Stuart	6.6	0.4	2.2	68.8	21.0	1.1	0.2
Seedling 1	4.3	...	3.9	69.2	21.2	1.3	0.3
Seedling 2	6.3	...	3.2	71.0	18.5	0.9	T
Curtis	6.1	...	3.1	71.8	18.1	0.8	0.1
Van Deman	5.1	T	2.9	74.6	16.0	0.9	T
Success	5.4	...	2.9	76.5	13.5	1.3	0.4
Other Oils							
Avocado, Taylor	19.1	7.1	0.4	55.8	17.0	0.8	....
Avocado, Lula	22.5	11.0	0.8	46.6	17.5	1.3	0.3
Citrus seed oil	28.0	0.3	5.4	22.8	37.2	6.5	....
Wesson oil[d]	22.1	1.1	2.7	17.1	55.6	0.2	0.1
Corn oil	12.0	...	2.1	29.7	54.8	1.2	0.2
Lard[e]	22.8	2.0	18.5	42.6	10.8	0.6	0.4

[a] Included in each peanut value: behenic 3.1%, lignoceric acid 1.1%, and U.S.M. 0.5%.
[b] Included 0.5% C_{15}.
[c] Trace.
[d] Not included 0.9% myristic.
[e] Not included 1.4% C_{15}.

eties are arranged in order of increasing oleic acid content. Oleic acid in the peanut oil ranges from 43 to 64% and in the pecan oil from 51 to 77%, with linoleic acid in complementary amounts of 33 to 18% and 38 to 14%. Saturated acids are nearly constant in level except for palmitic in the peanut. In the peanut oils palmitic acid ranges from 13 to 7% and varies directly with the linoleic acid content ($r = 0.92$) as indicated by the rank difference method.

The GLC procedure did not determine any acid containing above 20 carbons in its chain or take account of unsaponifiable matter. Iodine values, calculated from GLC data, were higher than the determined ones in the peanut oils. These values suggested the presence of significant quantities of saturated acids with chains longer than 20 carbons, so adjustment was made in the peanut oils percentage for a content of behenic (3.1%) and lignoceric (1.1%) acids, given as average values in Hilditch (1a). The peanut oils analyzed an average 0.5% unsaponifiable matter. These values totaled 4.7% which was applied equally to all peanut analyses.

Oleic acid in the two avocado oils analyzed 47 and 56% and linoleic acid remained at the 17 to 17.5% level. The acid complementary to oleic was hexadecenoic acid at 11 and 7% while palmitic acid varied directly with it from 23 to 19%.

Traces of components other than those mentioned above were found in certain of the oils. Traces of myristic and a 15-carbon acid were found in peanut and avocado oils. Trace of a 15-carbon but no 14-carbon acid was found in pecan oil. The rancid lard sample showed 14 pips between the 14-carbon and 20-carbon instead of the expected 7. Peanuts showed consistently slightly more arachidic content than the other oils and, judging from the agreement in the determined and calculated iodine values, these peanuts contained usual amounts of the longer chain saturated fatty acids: behenic and lignoceric.

TABLE II
Comparison of Fatty Acid Analysis by GLC and UV Absorption Following Alkaline Isomerization

Item	Oleic Acid, %		Linoleic Acid, %		Linolenic Acid, %		Iodine Value	
	GLC	UV	GLC	UV	GLC	UV	GLC[b]	Wijs
Peanut								
Spanish 18-38-47	43.1	40.7	32.5	32.7	1.5	0.2	97.5	101.8
NC 2	51.9	51.4	26.2	25.4	1.3	0.0	93.4	92.3
Va. Runner G 26	52.3	50.3	25.1	25.6	1.5	0.1	92.9	92.6
Spanette	52.5	50.9	25.7	25.2	1.3	0.1	93.4	92.0
Georgia 119-20	54.4	54.4	23.2	22.9	1.4	0.1	90.7	90.7
Va. 56 R	54.5	54.8	23.8	22.8	1.4	0.1	92.3	90.8
Early Runner	55.6	54.6	24.7	24.0	1.1	0.1	91.5	92.9
Virg. Bunch G2	55.7	55.3	23.7	22.8	1.3	0.2	92.4	91.3
Florigiant	55.7	55.1	22.9	22.2	1.6	0.2	91.8	90.3
Dixie Runner	58.5	57.1	22.4	21.8	1.1	0.1	92.0	91.1
Florida 393	63.5	61.6	17.7	17.4	1.4	0.2	88.9	87.4
Average	54.5	53.3	24.4	23.9	1.4	0.1	101.9	101.3
Pecan								
Moneymaker	51.0	51.3	37.8	37.0	1.7	1.8	114.0	[illegible]
Mobile	56.8	55.6	31.4	30.6	1.8	2.1	107.9	[illegible]
Big Z	59.7	62.7	30.3	29.3	1.6	1.4	108.0	[illegible]
Randall	61.3	59.5	28.2	27.4	1.5	1.5	105.2	[illegible]
Teche	63.4	62.1	25.7	25.2	1.3	1.4	102.4	[illegible]
Frotscher	63.9	62.9	25.5	25.4	1.3	1.5	102.5	[illegible]
Stuart	68.8	65.4	21.0	22.1	1.1	1.3	98.8	[illegible]
Seedling #1	69.2	69.2	21.2	20.4	1.3	1.4	99.6	[illegible]
Seedling #2	71.0	71.0	18.5	17.0	0.9	1.1	95.5	[illegible]
Curtis	71.8	70.5	18.1	17.7	0.8	1.0	95.9	[illegible]
Van Deman	74.6	73.6	16.0	15.0	0.9	1.1	94.2	[illegible]
Success	76.5	77.2	13.5	12.7	1.3	0.8	92.6	[illegible]
Average	65.7	65.1	23.9	23.3	1.3	1.3	101.4	[illegible]
Other Oils								
Citrus seed oil	22.9	24.7	37.2	35.1	6.5	6.7	100.2	101.1
Avocado, Taylor	62.8[a]	61.7	17.0	14.2	0.8	1.3	86.2	[illegible]
Avocado, Lula	57.6[a]	54.8	17.5	15.5	1.3	1.5	84.3	[illegible]
Wesson oil	18.2[a]	22.0	55.6	49.9	0.2	0.4	112.6	111.1
Corn oil	29.7	35.9	54.8	47.7	1.2	1.7	123.6	[illegible]
Lard	16.7[a]	61.3	10.8	2.5	0.6	1.1	61.1	[illegible]

[a] Includes hexadecenoic acid.
[b] Calculated.

The wide variation in unsaturated acid content of the pecans which were grown in this general area and the peanuts which were grown on the same farm under almost identical environmental temperatures suggested that genetic factors may be of importance equal to that of environmental temperature (1a) in influencing degree of unsaturation. Too, the data showed that in the peanut, possibly in the avocado, but not in the pecan, variation in palmitic acid correlated with variations in unsaturated acid content. These variations suggested that processes of synthesis of unsaturated and saturated fatty acid may not always be as separate in plants as had been hypothesized (1b).

It had been suggested that Spanish, runner, and Virginia types (5) of peanuts would show characteristic differences in concentrations of unsaturated fatty acids. The figures for unsaturated fatty acid concentrations showed little association with type. Perhaps this should be expected since a "pure type" might be hard to find today with geneticists making many crosses between types in order to produce peanuts with desired qualities. But it should be noted that 9 of the peanut oils were quite similar in composition, with one distinctly higher in linoleic acid and the other distinctly lower.

Comparative Analyses

Iodine values, Table II, as calculated from the GLC procedure, agreed well with those obtained in the Wijs procedure, except for the peanut oils. But the iodine values of peanut oils when adjusted for content of longer chain saturated fatty acids and for unsaponifiable matter, fell into line with the others except

possibly for Spanish 18, the peanut highest in linoleic acid.

Unsaturated fatty acid analysis by the two procedures shows surprisingly good agreement, Table II, except for Wesson oil, corn oil, and lard. With these samples bought in a local store, both oleic and linolenic acid have shown higher, and linoleic acid lower values, by GLC than by spectral methods. Craig and Murty (6) noted, with oils high in linoleic acid like corn, sunflower, and soybean, a similar lack of agreement in results. Interestingly, the slightly rancid leaf lard fitted into this same pattern. Herb *et al.* (7) demonstrated close agreement for lard by the GLC and UV methods and their analysis fitted our GLC pattern for lard. It is noteworthy that pecan oils (Table II) showed close agreement in linolenic acid content by the two methods while peanut oils did not, even though the linoleic acid ran through a similar range of values in the two series.

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FRACTIONAL BLOOD LIPIDS IN DOGS SUBJECTED TO PROLONGED FEEDING OF VARIOUS DIETS[1]

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INTRODUCTION

The problem of the relation between diet and blood lipid concentration has been approached frequently by the employment of studies on alimentary lipemia. Such studies have determined the variability of blood lipids over a few hours' time. It seemed, however, that the slow and irregular nature of the variability would lend itself more properly to a long term study. Consequently an experiment was planned in which normal dogs were to be fed diets high in various foodstuffs for one and half to six month periods. Fractional blood lipids were to be analyzed at ten to fourteen day intervals during these periods.

A few similar long term evaluations have been made. Page and Farr did not find any significant alteration of blood lipids in studies of nephrotic patients being fed various diets. Freyberg, Newburgh and Murrill found that diabetic patients on high fat diets had concentrations of blood lipids which were slightly lower than normal (rather than higher as one might expect). Corcoran and Rabinowitch, studying Eskimos on a high fat diet, also found decreased concentrations of blood lipids. Tolstoi, however, noted elevated levels of blood cholesterol in studies of two persons on a prolonged high protein and fat diet. Fleck, Corwin and one of us (Bollman) observed that when dogs were fed a high lard diet, hypercholesteremia developed only occasionally, and that there was usually depression of neutral fat values. However when adrenal lecithin was added to the diet, a regular rise of blood lipids occurred. Steiner and Domanski reported an experiment in which egg yolk (high in phospholipid) was fed to dogs. They noted a sustained increase of blood cholesterol averaging 203 mg. per 100 cc.

The quantity of food fed in such experiments should be an important consideration. It has now been shown that so called starvation hyperlipemia pertains only to short term studies and that in dog and man, at least, sustained undernutrition is accompanied by hypolipemia (Entenman and others, Man

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and Gildea, and Ling). Conversely, the stuffing or overfeeding of geese with rye meal produces an elevation of blood lipids (1, 6).

PROCEDURE

Thirteen adult mongrel dogs, five male and eight female, were used. Some were large, some small, some fat, some lean. They were weighed once a month, the largest weighing 21 kg., the smallest, 4.8 kg. Their activity was restricted to small individual cages. In the last four months of the experiment, record was kept of estrus in the females. The temperature of the kennel was kept about 65°F. during the winter, fall and spring. During the summer, the maximal outside temperature was 99°F.

The animals were fed once daily. Forced feeding was resorted to occasionally when the dogs refused to eat, as they sometimes did on the egg yolk and the lard diets. The quantity of food was kept constant during a specified period and was usually equivalent to one and a half times the basal requirement. The basal requirement was determined by calculating the surface area of each dog (12). A diet equivalent to one and a half times the basal requirement had been found adequate previously for maintenance of the body weight of dogs under similar circumstances. In some periods, however, the amount was altered so that a half, two, or two and a half times the basal requirement was given. This was designed to test the effect of the quantity of food fed on the concentration of blood lipids.

The diets used were mixed, high fat, high carbohydrate, high protein, and egg yolk. All but the last contained 4 per cent bone ash and 8 per cent canned tomatoes. The mixed diet contained 40 per cent lean raw horse meat, 40 per cent cracker crumbs and 8 per cent lard. Each 100 gm. of this diet contained 283 calories, of which 18 per cent were from protein, 40 per cent from fat and 42 per cent from carbohydrate. The high fat diet contained 40 per cent meat, 18 per cent cracker meal and 30 per cent lard. It yielded 396 calories per 100 gm., 10 per cent of which were from protein, 76 per cent from fat and 14 per cent from carbohydrate. The high carbohydrate diet was relatively low in protein and fat. It contained 30 per cent meat, 38 per cent cracker meal, and 20 per cent cane sugar, yielding 270 calories for each 100 gm., of which approximately 15 per cent were from protein, 14 per cent from fat and 71 per cent from carbohydrate. The high protein diet was low in carbohydrate and contained 88 per cent meat. Fifty-nine per cent of the caloric content was from protein, 40 per cent from fat and 1 per cent from carbohydrate, or 120 calories per 100 gm. of diet. The egg yolk diet consisted of mixed diet plus egg yolk. Powdered egg yolk was used for the first six months of the experiment, but after this, frozen egg yolk was substituted because of the unavailability of the dry product. Equal amounts of dried egg yolk and mixed diet were used to make the diet. Calculations revealed that it contained 480 calories per 100 gm. of diet, of

which 21 per cent were from protein, 12 per cent from carbohydrate and 67 per cent from fat. The dried egg yolk was analyzed; it contained 49.15 per cent alcohol-ether soluble material. Of this fraction 18.15 per cent was phospholipid and 4.15 per cent cholesterol. The frozen egg yolk was used in the ratio of two-thirds egg yolk to one-third mixed diet. This mixture proved to be more palatable to the dogs than the dried egg yolk mixture even though water had been added to the latter to make it more miscible.

The dogs were started on a six week control period on the mixed diet. During the rest of the eleven months they were shifted about from one diet to another at one and a half to six month intervals so that each dog received several types of diet. Diets were weighed daily for each dog. Blood specimens were taken every two or three weeks when the animal was in the fasting (twenty hour) state. Twenty-five cubic centimeter samples were removed from the external jugular vein. Heparin (0.2 cc. of 0.6 per cent solution) was added to the blood to inhibit clotting. The sample then was centrifuged at high speed for half an hour and the hematocrit reading was determined before the homogeneous plasma was pipetted off.

METHODS

Direct analyses of the plasma were made for total cholesterol, total phospholipid, choline-containing phospholipid and neutral fat. Values for cephalin and total fat were computed from the foregoing analyses but will not be included in this paper.

Aliquots for determinations of total cholesterol, total phospholipid and choline-containing phospholipid (lecithin and sphingomyelin) were taken from a 3:1 ethyl alcohol-ethyl ether extract of plasma. The solvent was mixed thoroughly with the plasma and extracted overnight at room temperature. Total cholesterol and total phospholipid were determined colorimetrically on a Cenco-Sheard-Sanford photelometer. A modification of the method of Bloor and his associates was used for the determination of cholesterol and a modification of Fiske and Subbarow's method was used for the determination of phospholipid. In the latter the alcohol-ether aliquot was not re-extracted with petroleum ether. Choline-containing phospholipids were determined according to Kirk's modification of Roman's titrimetric iodine procedure. Hydrolysis was effected by gaseous hydrogen chloride (8). Determinations of neutral fat were made according to the method of Voris, Ellis and Maynard. Duplicate determinations were made in all instances.

RESULTS

In a study such as this, definite conclusions are difficult to arrive at for the following reasons: 1. In studying variability of blood lipids a large group of

animals must be used. Therefore this group of thirteen animals can hardly be considered statistically significant. 2. At the outset, the hypothesis was adopted that we should compare the average blood lipid values on each special diet with the average values on the mixed diet even though the period when the mixed diet was given might have been nine months previous. It was found, however, that although some diets appeared to have a positive effect on the blood lipids of certain dogs, the actual average levels were not greatly different from those of the control period. For example, if the blood lipids had been

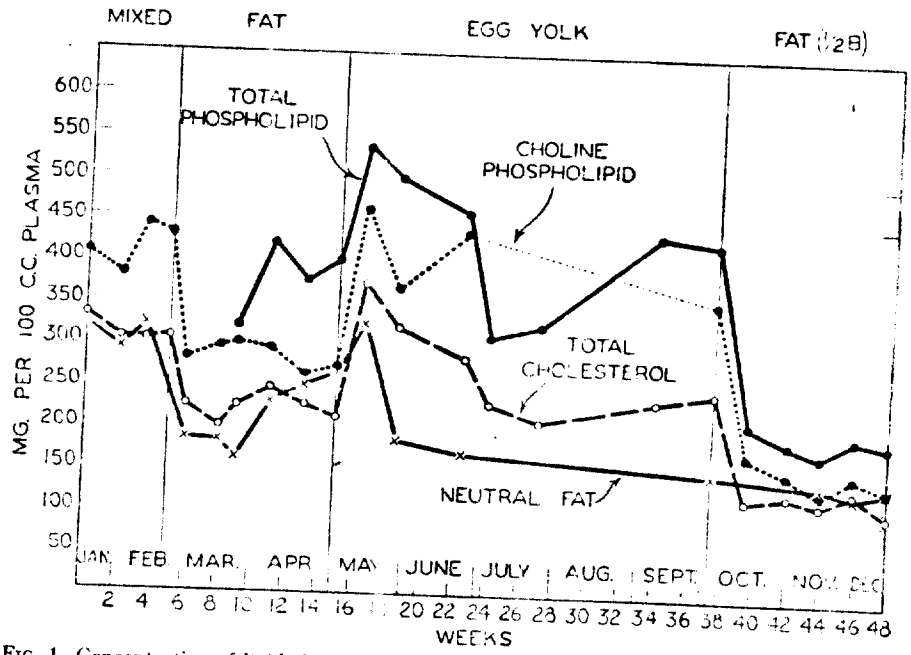

FIG. 1. Concentration of lipids in the plasma of a female dog that received mixed, fat and egg yolk diets equivalent to one and half times the basal requirement and a fat diet equivalent to half the basal requirement.

lowered by diet "A" and elevated by a subsequent diet, "B", that elevation, though seemingly definite, might not be any higher than normal values had been some months before. Nevertheless, an attempt was made to follow the original plan. Diets used for such comparison were always one and a half times the basal requirement. Those of greater or less amount were not included.

The most definite conclusion to be arrived at is that striking alteration of blood lipids of the dog by isocaloric alteration of diets is difficult to achieve, and that moderate changes are correspondingly difficult to evaluate. However, certain trends became apparent during the study. These are demonstrated in figure 1, which is the record of the blood lipids of one dog. One may note

thereon (1) a depression of all elements on a high lard diet, (2) a temporary elevation (several weeks) of phospholipid, choline phospholipid and cholesterol with the egg yolk diet, (3) a depression of all levels on a diet low in caloric value. Other trends not shown in figure 1 are a tendency toward temporary elevation of blood lipid elements with diets high in caloric content and the possible elevation of blood lipids at the period of estrus.

Mixed diet.—As a rule, levels for blood lipids of individual dogs were fairly constant when on the mixed diet, but these "normal" levels varied greatly from dog to dog. Three of the thirteen dogs had their highest values of the year on this diet, and three their lowest. Only one of the former group received the egg yolk diet in some other period. Two in the latter group received high lard diets at some subsequent date and one of these had equally low levels on the high lard diet.

High protein diet.—Four dogs received a diet high in protein. None had their highest or lowest values of the year on this diet. When a comparison was made between the blood lipid levels on the mixed and high protein diets, it was found that there was a slight (and perhaps insignificant) decrease of all fractional lipid elements on the high protein diet. The average mean differences[2] for the whole period were −32 mg. per 100 c.c. of plasma for cholesterol, −26 mg. per 100 c.c. for choline phospholipid and −70 mg. per 100 c.c. for total phospholipid. (The last figure may be misleading because of the paucity of normal values available for comparison.)

High carbohydrate diet.—Of the four dogs receiving the high carbohydrate diet, one had its lowest and one its highest blood lipid values of the year on this diet. Average mean differences between mixed and carbohydrate diets were not impressive, actual values being −14 mg. per 100 c.c. for cholesterol, 15 mg. per 100 c.c. for choline phospholipid and 14 mg. per 100 c.c. for total phospholipid. All in all, there was little difference between mixed and high carbohydrate diets.

Egg yolk diet.—Seven dogs received an egg yolk diet equivalent to one and a half times the basal requirement (the amount necessary for maintenance of the body weight). Five of these had their highest values of the year on this regimen. In the other two dogs values at no time rose above control levels. Two dogs received a diet equivalent to two and a half times the basal requirement and got a more prominent elevation than one would have expected on a diet equivalent to less than one and a half times the basal requirement. Two dogs received diets equivalent to less than one and a half times the basal requirement. In these dogs there was no change of levels from control values. When the averages of the egg yolk diet were compared with the "control" figures,

[2] A comparison of the averages on the special diet with those of the mixed diet of isocaloric value was made. The differences [illegible] for each dog were averaged, and the resultant figure was taken to represent the [illegible] differences."

however, there was little noticeable difference between the two, the concentration of cholesterol being +7 mg. per 100 c.c., for choline phospholipids +47

TABLE 1

Plasma lipid values after administration of isocaloric egg yolk diets

DOG	LAST VALUES ON PREVIOUS DIET	EGG YOLK DIET			
		2 weeks	4 weeks	8 weeks	Final
			Total phospholipid		
	mg. per 100 cc.				
2	296	320	323		397 (15th wk.)
9	342	430	425	596	467 (22nd wk.)
12	375	467	487	625	495 (22nd wk.)
8	400	535	500	458	422 (22nd wk.)
10	410	531	480	452	448 (10th wk.)
			Choline-containing phospholipid		
9	258	380	333	553	397 (22nd wk.)
13	265	342	289	278	320 (32nd wk.)
12	269	370	368	517	438 (22nd wk.)
8	272	462	368	437	354 (22nd wk.)
2	278				375 (14th wk.)
10	342	462	484	436	427 (10th wk.)
3	392		458		
10	439	364	451	470	447 (10th wk.)
			Cholesterol		
13	184	232	184	244	176 (32nd wk.)
2	192	266	223		260 (15th wk.)
9	200	268	268	340	264 (22nd wk.)
10	208	356	390	367	339 (10th wk.)
8	228	372	320	284	244 (22nd wk.)
12	228	332	320	436	340 (22nd wk.)
3	292	420	412		
10	408	304	336	412	416 (10th wk.)
			Neutral fat		
2	73				142 (15th wk.)
10	184			255	279 (10th wk.)
9	188	243	144	148	95 (22nd wk.)
12	197	248	155	179	142 (22nd wk.)
8	270	324	183	170	179 (22nd wk.)
13	273	231	112	205	137 (32nd wk.)
10	279	251	229	280	252 (10th wk.)
3	353	295	252		

mg. per 100 c.c., for total phospholipids +8 mg. per 100 c.c., and for neutral fat −52 mg. per 100 c.c. And yet there remained the impression that egg yolk

elevated the cholesterol and phospholipid fractions. As the "control" figures had been taken as long as nine months previously, it was thought that a new slant might be obtained by comparing blood lipid values on the diet preceding the egg yolk with those of the egg yolk diet. When this was done (comparing the last value on the previous diet with the average figures on the egg yolk diet) it was found that the total phospholipid averaged 71 mg. per 100 c.c. higher, the choline phospholipid 79 mg. per 100 c.c. higher, the cholesterol 58 mg. per 100 c.c. higher and the neutral fat 34 mg. per 100 c.c. lower (table 1). The rise on egg yolk diets was not sustained but tended to reach its peak at the second to the eighth week and to fall gradually thereafter.

Two dogs were put on a high egg yolk diet equivalent to two and a half times the basal requirement. They gained weight on this diet. Both had a prompt but variably sustained increase of the concentration of lipids. The average elevation in the two dogs from the averages of the preceding diet was +152 mg. per 100 c.c. for cholesterol, +135 mg. per 100 c.c. for total phospholipid, +167 mg. per 100 c.c. for choline phospholipid and +143 mg. per 100 c.c. for the neutral fat. When compared with the averages on the mixed diet the elevations were not so positive but showed increases of +107 mg. per 100 c.c. for cholesterol, +132 mg. per 100 c.c. for choline phospholipid and +89 mg. per 100 c.c. for neutral fat. Figures for total phospholipid were not available.

Lard diet.—Ten dogs received high lard diets; seven had their lowest and one the highest levels of the year on this regimen. Generally, the plasma lipid values fell within two weeks after the dogs began eating the diet and remained low for the duration of the period. This persistence of slightly lowered levels is reflected in the average mean differences between mixed and high lard diets, as cholesterol was 77 mg. per 100 c.c. lower, choline phospholipid 52 mg. per 100 c.c. lower and total phospholipid 89 mg. per 100 c.c. lower than on the mixed diet. One of the dogs on a high lard diet equivalent to half the basal requirement had the lowest values encountered in any dog throughout the year. Its values on the low calorie-high lard diet averaged 191 mg. per 100 c.c. lower for cholesterol, 256 mg. per 100 c.c. lower for choline phospholipid and 178 mg. per 100 c.c. lower for neutral fat. (Control figures for total phospholipid were not available for comparison.) Conversely, overfeeding with lard diet in one dog resulted in a temporary increase (with subsequent decrease) of plasma lipid levels.

Individual plasma lipids.—As a general rule, the cholesterol, total phospholipid and choline phospholipids tended to parallel one another, while the neutral fat values varied irrespective of the type of diet used. The total phospholipid usually showed the greatest variation of the first three types, and the cholesterol the least. Duplicate checks with the neutral fat method were often 20 per cent or more at variance.

In summary, the lard diet and the egg yolk diet seemed to affect the plasma

lipids of the dog in opposite directions, the former causing a decrease and the latter a temporary increase. Yet the fat content of these diets was surprisingly similar (76 per cent of the calories of the lard diet were derived from fat, and 67 per cent of the calories of the egg yolk diet were from fat). We are left with the hypothesis that if such trends are real, the type of lipid in the diet must have an influence in plasma lipid levels, for the type which is high in phospholipid may cause an increase of plasma lipids and the type which is high in neutral fat may cause a decrease.[3]

CONCLUSIONS

1. Prolonged administration (one and a half to six months) to normal dogs of diets rich in protein, carbohydrate, lard or egg yolk, rarely produces much change of plasma lipid concentration.

2. A temporary elevation (two or more weeks) of plasma cholesterol, choline phospholipid and total phospholipid results with administration of high egg yolk diets. This elevation is greater when the quantity of food fed is increased.

3. A moderate sustained depression of plasma lipid fractions usually results with diets high in lard. This depression is greater when the quantity of food fed is diminished.

4. Diets high in protein or in carbohydrate cause little change of plasma lipids.

5. Total phospholipids, choline phospholipids and cholesterol are the most reliable indexes of variability of plasma lipids.

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[3] In three of four dogs observed in estrus, there seemed to be an elevation of plasma lipids near the period time. In one the changes were extreme. In the fourth dog there was no change, but the dog was on a low caloric diet at the time and its plasma lipids were the lowest of any dog of the year.

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Foreign-Body Type Reaction in Fat Cells.* (24039)

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During studies of the response of rats to diets containing very high amounts of saturated fats, a foreign-body type reaction was observed in fat cells of adipose tissue adjacent to sections of various organs prepared for histological examination. This report describes these altered fat cells and various dietary conditions under which they occur. Although a similar abnormality had been observed earlier with one of these fats by Cox and DeEds,† their data have not been published.

Methods and materials. Weanling male and female rats were obtained from Holtzman Rat Co. and fed one of the following diets. Each diet contained 20% vitamin-test casein, 4% B-vitaminized casein(1), 5% USP XIV salts, 4% corn oil, 17% glucose, and supplements of Vit. A, D, and E at levels of 40,000 I.U., 4,000 I.U., and 510 I.U./kg diet, respectively. The saturated fats incorporated at a level of 50% were fully-hydrogenated lard, palmitic acid, stearic acid, or a saturated partially acetylated monoglyceride(1). Ethyl stearate was fed at 45% with 5% cellulose added to provide body to the diet. A control group was given 50% lard. Rats were sacrificed at 8-week intervals and examined grossly. Portions of perigastric, perirenal, periadrenal, perigonadal, and subcutaneous fat were removed from each rat, fixed with Bouin's solution, sectioned, and stained with hematoxylin and eosin.

Results. Gross examination of the viscera and body cavities was not remarkable except for general fullness of the gastrointestinal tract with creamy colored food and an occasional mottled appearance of fat depots in animals fed the saturated fats.

Microscopic examination of sections showed an unusual foreign-body type reaction in fat from rats fed saturated fats (Fig. 1, top). The lesion consisted of a multinucleated cellular reaction usually located within the fat cell membrane with nuclei arranged evenly about periphery of the fat cell or in clumps. In many instances, the material which occupied the center of the fat cell, appeared to have been partially dissolved, leaving delicate slit-like spaces which were radially oriented and suggestive of fatty acid crystals (Fig. 1, bottom). The reactive process usually involved 2 or more adjacent fat cells and was separated from similar areas by uninvolved fat cells. No acute inflammatory reaction, evidence of recent hemorrhage, or birefringent material were associated with the fat reaction.

The phenomenon occurred within 8 weeks in rats fed diets containing palmitic acid, stearic acid, ethyl stearate, or saturated partially acetylated monoglycerides and within 16 weeks in rats fed the fully-hydrogenated lard diet, but was not observed in rats fed the control lard diet. The flecks of opaque white material observed grossly in the depot fat of some rats were associated with the severest occurrence of the foreign-body type reaction as seen microscopically.

* Communication No. 247 from Research Labs., Distillation Products Industries.

† Observed in rats fed saturated acetylated monoglycerides for long periods of time by A. J. Cox, Dept. of Pathology, Stanford Univ., Calif., and F. DeEds, Western Regional Research Laboratory, Albany, Calif., private communication. *Added in proof:* Recently published by Cox and DeEds, *Am. J. Path.*, 1958, v34, 263.

FIG. 1. *Top:* Periadrenal fat showing multiple islands of fat injury separated by normal fat. Hematoxylin and eosin, ×60. *Bottom:* Higher magnification showing variations of cellular reaction. Multinucleated giant cells are present at top of print. A fat cell containing radial slit-like spaces is seen near bottom of print. Hematoxylin and eosin, ×430.

This reaction in visceral and retroperitoneal fat of the rat bears some histologic resemblance to pathologic conditions of human fat such as fat necrosis of newborn(2), sclerema neonatorum(3), and traumatic fat necrosis of various types(4,5,6). Further studies are underway to determine the histochemical characteristics of the lesion.

Appearance of the foreign-body type reaction in all groups except that fed lard indicates that the reaction is a response to a relative or absolute excess of saturated long chain fatty acids. We are testing diets containing various proportions of saturated to unsaturated fatty acid to determine the critical ratio which produces this reaction.

Summary. A foreign-body type reaction has been observed in fat from rats fed diets containing high levels of saturated fat. The phenomenon and the dietary conditions under which it was produced, are described.

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Effect of Dilantin Sodium on Tensile Strength of Healing Wounds.* (24040)

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Hyperplasia of the oral gingiva is a common reaction among epileptics following systemic administration of sodium diphenylhydantoinate (Dilantin Sodium). The phenomenon does not occur in all patients and, in those affected, there is a wide range in severity of the lesion(1,2). The reason for this selective involvement of gingival tissues, other tissues throughout the body apparently being unaffected, is unknown nor is the mechanism of hyperplasia understood. Tissue reaction is basically a fibrous hyperplasia with formation of increased bulk of dense collagen fibers. This has suggested to Shapiro that the compound might favorably influence the healing of gingival wounds through enhancing collagenization(3). He reported that administration of dilantin to non-epileptic patients in-

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Experimental Diabetes and Diet

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The influence of different diets on sensitiveness to alloxan and experimental pancreatic diabetes in the white rat has been investigated.

Two hundred twenty-three female rats weighing between 90 and 120 grams were divided into several groups and fed different diets for a period of one month. At the end of the month and 12 hours after the last meal they were injected intraperitoneally with 160 mg./kg. of alloxan. The mortality rate was considered during one week after the injection, and in all cases high hyperglycemia preceded death.

The results were as follows (see Table 1):

(1) In the group given a high carbohydrate diet the mortality due to alloxan was 40 per cent.

(2) In the group given a low protein diet (10 per cent casein content) there was 90 per cent mortality. Increasing the protein content from 20 to 45 per cent increased the resistance to alloxan (40 and 33 per cent mortality).

TABLE 1

Diets	Wheat flour (%)	Corn flour (%)	Casein (%)	Fat (%)	Yeast (%)	Cod liver oil (%)	Salt mixture (McCollum Davis) (%)	No. of rats used	Mortality (%)
(a) High carbohydrate	35	34	20	0	5	5	1	15	40
(b) High protein	10	34	45	0	5	5	1	15	33
(c) Low protein	40	39	10	0	5	5	1	15	90
(d) High lard	10	25	20	34	5	5	1	20	100
(e) High lard and high protein	14	15	30	30	5	5	1	15	100
(f) High ox fat	10	25	20	34	5	5	1	14	86
(g) High lard, and methionine (200 mg./kg./day)	10	25	20	34	5	5	1	10	30
(h) High lard, and sulfanilamide (250 mg./kg./day)	10	25	20	34	5	5	1	10	90
(i) High lard, and choline (300 mg./kg./day)	10	25	20	34	5	5	1	15	100
(j) High lard, and thiouracil (200 mg./kg./day)	10	25	20	34	5	5	1	10	10
(k) High olive oil	10	25	20	34	5	5	1	15	40
(l) High butter	10	25	20	34	5	5	1	15	33
(m) High oleomargarine	10	25	20	34	5	5	1	15	21
(n) High corn oil	10	25	20	34	5	5	1	15	13
(o) High coconut oil	10	25	20	34	5	5	1	14	0
(p) High lard (30%) and coconut oil (10%)	9	20	20	40	5	5	1	10	20

(3) High lard and high ox fat diets decreased strongly the resistance to alloxan (100 and 86 per cent mortality, respectively). This effect was not modified in the former either by increasing the casein content from 20 to 30 per cent (100 per cent mortality) or by treatment with choline or sulfanilamide. In contrast, the treatment with methionine or thiouracil corrected the hypersensitiveness (30 and 10 per cent mortality, respectively).

(4) The sensitiveness to alloxan was not modified when high olive oil or high butter diets were administered (40 and 33 per cent mortality, respectively), but there was an increased resistance to alloxan in the rats fed either a high oleomargarine diet (21 per cent mortality) or in those fed a high corn oil diet (13 per cent mortality).

(5) Complete protection to alloxan in rats fed a high coconut oil diet (0 per cent mortality) was observed.

(6) In rats fed a high lard diet modified by the addition of 10 per cent coconut oil, a partial protection to alloxan was observed (20 per cent mortality).

The role of the quality of diets and the amount of food fed on the initiation and evolution of experimental pancreatic diabetes in rats has also been studied.

Fifty-one male rats weighing about 130 grams were subtotally pancreatectomized (95 per cent of the pancreas) and

* The thymine used in this work was chemically pure and found to have 280 γ of folic acid activity/gram.

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...ided into three groups, each of which was fed one of the ...ts shown in Table 1 (a, b, and d).

As shown in Table 2, the high protein diet showed a pro...ctive effect and the high lard diet an unfavorable effect in ...parison with that which was high in carbohydrate.

TABLE 2

...e after ...ration ...s.)	High protein diet		High carbohydrate diet		High fat diet	
	Diabetes		Diabetes		Diabetes	
	Total	%	Total	%	Total	%
1	4/16	25	2/18	11.1	7/17	41.1
2	9/16	56.2	14/18	77.7	17/17	100
4	10/16	62.5	15/17	88.2	15/15	100
5	10/16	62.5	12/13	92.3	15/15	100
6	11/15	73.3	11/12	91.6	14/14	100
7	11/15	73.3	11/11	100	10/10	100
8	9/12	75.0	10/10	100	5/5	100

...a overfed rats (fed three times daily with a high carbo...rate diet) the evolution of diabetes was much faster than ...se fed once a day and eating less food. Furthermore, the ...r diet but restricted in quantity (about 80 per cent of the ...ent of food eaten by a normal rat) produced a marked ...n in the time of initiation of diabetes (see Table 3).

TABLE 3

...e after ...ration ...s.)	Underfed		Overfed		Ad libitum	
	Diabetes		Diabetes		Diabetes	
	Total	%	Total	%	Total	%
1	1/13	8	4/17	23.5	2/18	11.1
2	5/12	41.6	15/15	100	14/18	77.7
4	3/7	42.8	10/10	100	15/17	88.2
5	1/7	14.3	7/7	100	12/13	92.3
6	4/7	57.1	5/5	100	11/12	91.6
7	3/7	42.8	3/3	100	11/11	100
8	3/7	42.8	3/3	100	10/10	100

...etes appeared first in those rats which received their ...nce a day (in a quantity proportional to their body ... rather than in those receiving the same amount of ...vided into three feedings at 7-hour intervals. It may ...osed that the higher postprandial hyperglycemia of the ... diet exerts an unfavorable effect, whereas the post...al hyperglycemia resulting from the latter is less ...

Summary

...e experiments described the following observations ...en made:

The toxic and diabetogenic action of alloxan increases ...d a low protein diet and even more so in the case of a ...or ox fat diet.

The action of these fat diets was corrected by the ... of methionine, thiouracil, or coconut oil, but there was no modification either by addition of choline or sulfonamide or by increase in the protein proportion.

(3) In rats fed other high fat diets (olive oil, butter) the actions of alloxan were not modified, but there was a slight diminution when high oleomargarine or corn oil diets were fed. However, complete protection was observed when a high coconut oil diet was administered.

(4) The unfavorable effect of the high lard diet was observed also on the initiation and evolution of diabetes due to subtotal pancreatectomy. Contrarily, feeding a high protein diet and especially treatment with thiouracil had a favorable effect.

(5) Diabetes appeared first in subtotal pancreatectomized rats which were overfed, then in those which ate ad libitum, and finally in those which were underfed. Diabetes appeared in rats fed a single meal before it did in those eating the same amount of food divided into three meals.

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The Monoglyceride Pathway of Fat Absorption in Man *

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Abstract. The absorption of fat was studied in five male subjects with cannulation of the thoracic duct in the neck by the administration of doubly labeled monoglycerides, or triglyceride as well as labeled free glycerol or labeled free oleic acid, by gastric or duodenal intubation.

Total recoveries of the administered glyceride radioactivity from the lymph lipids ranged from 35 to 53% for the glycerol label (tritium) and from 35 to 57% for the fatty acid label (^{14}C). The recovery of administered radioactive free glycerol in lymph lipids was only 4.1%, even when given in mixture with bile salts, fatty acid, and monoglyceride.

A comparison of the isotope ratios of the two components (glycerol and fatty acid) of the lymph glycerides with the ratios of these components of the original meal glyceride showed little change during the initial period of fat absorption, indicating that the doubly labeled monoglycerides passed into the lymph intact. During the later part of the period of major fat absorption, the ratios in lymph lipids changed due to loss of glycerol representation, indicating monoglyceride hydrolysis and portal venous diversion of free glycerol.

Confirmation of the intact nature of 2-monoglyceride during absorption was made by analyzing the amount and position of the labeled fatty acid in the lymph triglycerides. The percentage of labeled fatty acid in the various positions of the lymph triglycerides was virtually identical with that of the meal during the initial period of fat absorption and then changed reflecting isomerization of fatty acids and subsequent complete hydrolysis of the glycerides.

The 2-monoglyceride pathway appears to be the major route of fat absorption for man during normal digestion and absorption of dietary triglyceride.

Introduction

The major portion of dietary fat is insoluble in water and important changes take place in the physicochemical state of dietary triglycerides in the gut lumen before absorption into the intestinal epithelium. Complete hydrolysis of triglyceride yields free fatty acids and glycerol; partial hydrolysis results in free fatty acids, monoglycerides, and diglycerides. Recently, it was shown in man that monoglycerides, free fatty acids, and conjugated bile salts readily form ultrafine aggregates or micellar solutions, from which fatty acids and monoglycerides may be absorbed into the intestinal mucosal cells (2). In whole animal studies, in which the glycerol and fatty acid moieties of glycerides were labeled with different isotopes, it was demonstrated (3-5) that 2-monoglycerides remain largely intact during intestinal absorption and reesterification into chylomicron triglycerides. This

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direct acylation pathway of monoglycerides to higher glycerides appears to be uniquely active in intestinal tissue, and is not appreciably demonstrable in other sites of triglycerides synthesis, such as the liver (6).

The present studies were undertaken to investigate glyceride absorption in man by administering singly and doubly labeled lipid compounds and analyzing in the subsequent hours lymph obtained by thoracic duct cannulation. The particular goal was to determine whether the monoglyceride pathway that has been shown in animals is also present in man and to attempt to assess its quantitative importance during normal fat absorption. The results of these studies suggest that the monoglyceride pathway is the predominant one during normal fat absorption.

Methods

Materials. The test glycerides were 2-^{3}H-glycerol-monostearate-1'-^{14}C in which the stearic acid was in the 1- or 2-position and 2-^{3}H-glycerol-1,3-dioleate-2-oleate-1'-^{14}C. The monostearin was synthesized from stearic acid-1-^{14}C and glycerol-2-^{3}H obtained from New England Nuclear Corp., Boston, Mass. Carrier stearic acid (Applied Science Laboratories Inc., State College, Pa.) was 99.8% pure (gas-liquid chromatography) and glycerol (Merck) was freshly redistilled at 2 mm Hg pressure. The method of preparation of 2-monostearin has been previously described (7). The final product had a specific activity of 30 μc/mg for ^{3}H and 15 μc/mg for ^{14}C. On hydroxylapatite thin-layer chromatography it moved as a single spot from which 98% of the radioactivity could be recovered. Saponification of the eluted material from the spot and partition between petroleum ether and acidified water phases showed that all the measurable ^{14}C activity was in the fatty acid and the ^{3}H in the glycerol moiety. The doubly labeled 2-monostearin was stored dry at −20°C but nevertheless showed gradual isomerization spontaneously to the 1-isomer. At the time of use in Study I, the material assayed 70% 2-monostearin, 30% 1-monostearin. The preparation of the micellar solution in Study II involved warming and some alkalinization, which appeared to enhance the isomerization so that assay of the final product instilled showed 20% 2-monostearin and 80% 1-monostearin.

The labeled triolein was prepared from oleic acid-1-^{14}C and glycerol-2-^{3}H, as previously described (7). For these studies a mixture of 2-^{3}H-glycerol trioleate and glycerol-1, 3-dioleate-2-oleate-1'-^{14}C in the ratio of approximately 10:1 provided a specific activity of 1.6 μc/mg for ^{3}H and 0.16 μc/mg for ^{14}C. Verification of the purity of these glycerides was carried out frequently over the course of many months by thin-layer and gas-liquid chromatography and by enzymatic hydrolysis. The purity of the glycerides was within 4% of the indicated composition of each compound at the time of each study.

Experimental design. In each instance the test glyceride was administered after a 10-12 hr fast. Patients were studied in the Clinical Research Center of New York University School of Medicine at Bellevue Hospital. Five male patients were the subjects for this study. Thoracic duct cannulation was carried out in four of the patients for therapeutic or diagnostic reasons related to their primary disorder; one patient, W.H., was a volunteer.

B.B. was a 50 yr old Negro male with a history of rheumatoid arthritis for 3 yr before study. Thoracic duct cannulation was performed for study of rheumatoid factor in lymph. The test meal for this patient was 120 mg of a mixture of 1- and 2-isomers, in a ratio of 30:70, of doubly labeled monostearin made into micellar solution with 2 ml of Emulphor EL 620,[1] and 23 ml of carbon dioxide-free water at 40°C. 20 ml of the solution was instilled via Miller-Abbott tube into the duodenum, and the tube flushed with warmed water and withdrawn. 2 hr later the patient was given 100 g of beef, and 200 ml of fruit juice; 3 hr later he began a regular diet and was encouraged to take fluids. Egg nogs were frequently given to maintain fluid and caloric balance; the marked variations in triglyceride content of the lymph samples reflect both fluid and fat content of the diet. Collections were continued for 48 hr.

J.K. was a 73 yr old white man with known hypertension for 3 yr. Admission to the hospital was prompted by abdominal pain. Exploratory laparotomy revealed a large retroperitoneal lymphosarcoma. It was planned to treat this tumor by retrograde instillation of streptonigrin into the thoracic duct. The monoglyceride study was carried out 2 days after cannulation and before any anti-tumor therapy. The test meal for this patient was 180 mg of a mixture of 1- and 2-isomers, in a ratio of 80:20, of doubly labeled monostearin in a micellar solution; the monostearin was dissolved in 4 ml of ethyl ether to which 0.5 g of oleic acid dissolved in 3 ml of ethyl alcohol was added. Pure sodium taurocholate, 0.5 g, was dissolved in 20 ml of warmed phosphate-buffered saline solution (15 ml of 0.01 M NaH_2PO_4, 30 ml of 0.01 M Na_2HPO_4, and 40 ml of 0.45% NaCl). The bile salt phosphate buffer mixture was added to the warmed ether alcohol fatty acid-monostearin mixture with continued mixing and heating until ether odor was no longer detectable. The initial pH was 6 and NaOH (1 N) was added until the pH was 7.2; the solution was initially cloudy but became water clear with formation of a stable micellar solution at pH 7.2. 20 ml of this solution was introduced into the duodenum and the tube was washed with 15 ml of additional warm water and withdrawn. Regular feedings were started 6 hr later. These also included egg nogs which markedly altered the flow and fat content of the lymph. The cannula became occluded 16 hr after feeding of the test meal, and no additional lymph was collected. Radioactivity was noted in the 2 and 6 hr samples.

[1] Emulphor EL 620, polyoxyethylene triricinoleate, General Aniline & Film Corporation, New York, N. Y.

W.H. was a 55 yr old white man admitted because of purpura and peripheral vascular disease. Extensive hematologic study failed to reveal any cause of the purpura. Thoracic duct cannulation was carried out for study of labeled triolein absorption. Duodenal intubation was unsuccessful in this patient. 1 hr before the administration of the radioactive meal, 40 g of triolein and 100 ml of fruit juice were given by mouth. The labeled triolein was mixed with aspirated gastric contents and reinjected into the gastric tube. Additional warmed water was given and the tube withdrawn. Fluids were given 2 hr later and regular meals 6 hr later. Egg nogs were used as supplementary feedings. Collections via cannula were continued for 36 hr. Virtually no radioactivity was recovered in the 0–2 hr collection after feeding. Radioactivity was detected in plasma samples at 2 hr after the test meal.

E.G., a 59 yr old white man, was admitted because of a recurrent pulmonary infection associated with his chronic pulmonary disease. Thoracic duct cannulation was performed at the time of a supraclavicular lymph node biopsy; the thoracic duct lymph and lymph node were studied for acid fast infection, neoplasm, granulomata, and fungi. The labeled triolein study was carried out on the first afternoon after cannulation; the study of the absorption of labeled free oleic acid and unlabeled triolein was performed on the 3rd day after cannulation and 42 hr after the labeled triolein study. The patient was given 30 g of unlabeled triolein mixed with fruit juice. One-half hour later, intestinal contents were aspirated from a tube previously passed into the duodenum. About 7 ml of the intestinal aspirate was mixed with the doubly labeled triolein and 0.5 ml of unlabeled triolein, and a homogeneous emulsion was formed by sonication with a BioSonik Model II Sonicator (Bronwill Scientific, Division of Will Scientific, Inc., Rochester N. Y.) for 30 sec. This material was introduced into the tube and the tube flushed with tap water. The tube was withdrawn 30 min later. The patient received a normal diet 2.5 hr after the triolein; lymph samples were continually collected from the time of surgery for 42 hr. Radioactivity was noted in the 6 hr sample. The labeled oleic acid unlabeled triolein study (Study V) was begun, in this patient, when radioactivity in lymph samples had fallen to a low level. 20 ml of unlabeled triolein and 50 μc of oleic acid-1-^{14}C (specific activity 24.6 mc/mm) were mixed with fruit juice and taken orally by the patient. During the 16 hr before the test meal solid food had been withheld but fluids were freely given. The patient's usual diet was resumed 1½ hr after the labeled oleic acid unlabeled triolein mixture had been ingested.

S.P., a 36 yr old white man, was admitted because of chronic alcoholism for 16 yr, and ascites for 2 months. Cirrhosis was obtained after salt restriction, and abdominal paracentesis of 1 liter. Thoracic duct cannulation was carried out to assay pancreatic enzyme activity in thoracic duct lymph. The radioactive glycerol study was carried out on the 4th day after cannulation. Glycerol ^{14}C (40 μc) obtained from Nuclear-Chicago was mixed with 10 ml of warm water. A micellar solution was made as follows. Na taurocholate (0.484 g and Na glycocholate 1.288 g (Nutritional Biochemicals Corp., Cleveland, Ohio) were dissolved in phosphate buffer (0.077 M NaCl; 0.045 M NaH_2PO_4). Monoolein 0.56 g, heptadecanoic acid 1.025 g, palmitic acid 0.96 g, and unlabeled glycerol 0.138 g were dissolved in ethyl alcohol: ethyl ether mixture 3:5 (v/v). The buffered bile salts were added to the ether-alcohol lipid solution with continuous stirring and heated to 45°C. The solution (total volume 150 ml) was clarified by the addition of NaOH to pH 7.1. The solution was administered by intraduodenal tube during a 45 min period; the 10 ml of radioactive glycerol solution was admixed with the micellar solution at 35 min after start of instillation, and the tube flushed with additional nonradioactive solution.

Analytic procedures. Lymph samples were collected into iced containers and stored at 4°C until analyzed. The samples were strained through gauze, the volumes recorded, and an appropriate fraction extracted with 20 volumes of chloroform: methanol 2:1 (v/v). Separation into two layers was achieved by either large volumes of acid water (0.05% H_2SO_4) or by smaller volumes of the dilute acid with centrifugation and washing of the chloroform fraction three times (8). Chloroform extracts were taken to dryness, and scintillant solution (2,5 diphenyloxazole, 4 g per liter of toluene, and 1,4-bis[2-(4-methyl-5-phenyloxazolyl)] benzene 100 mg per liter of toluene) was added. Samples were counted in a Tri-Carb (Packard) liquid scintillation spectrometer. Correction was made for isotope overlap in each channel; disintegrations per minute were calculated from efficiency data using known standards. Samples of the original meal were extracted in similar fashion and counted. Plasma samples were treated similarly. Quenching activity was approximately the same for all samples.

Triglyceride analysis was carried out on extracts of lymph by the method of Van Handel and Zilversmit (9); total lipids were estimated by the potassium dichromate method of Bragdon (10). Chylomicron fractions were prepared from lymph samples by centrifugation in a Spinco Model L ultracentrifuge at 25,000 rpm, 15°C either in a No. 40 rotor or in a No. 20 rotor. The creamy top layer was suspended in buffered phosphate-EDTA solutions and respun as previously described by Kayden, Karmen, and Dumont (11). Lymph and chylomicron fractions were separated by thin layer chromatography on Silica Gel G (Brinkmann) plates using a solvent system of petroleum ether: ethyl ether: glacial acetic acid 70:30:0.8. Standards and unknowns were made visible by iodine vapor. Fractions of cholesterol ester, free fatty acids, glycerides, and phospholipids were scraped from the plate and either counted directly with scintillant solution, or extracted with chloroform and methanol, dried, and counted with scintillant solution in the liquid scintillation spectrometer.

Identification of the amount of labeled fatty acid present in the 1- and 3 positions in comparison with the 2 position was accomplished by enzymatic hydrolysis of the isolated triglyceride fraction, which was separated from other lipids (12) by silicic acid column chromatography.

Results

Study I, B.B., Table I. Total recovery of each isotope was 53% ^{3}H and 57% ^{14}C. Most (96%) of the total recovered ^{14}C-fatty acid was collected in the 8 hr period after feeding. During this period of absorption, the ratio of lymph isotopes $^{3}H/^{14}C$ to meal isotopes $^{3}H/^{14}C$ was initially 1.05, then 0.95, and then fell to 0.69 (Fig. 1). The fraction of fatty acid label in the 2 position was initially very similar to that of the test meal, but then decreased as absorption continued.

Study II, J. K., Table I. Total recovery of each isotope was 35% ^{3}H and 49% ^{14}C. The major portion (96%) of the total ^{14}C-fatty acid recovered was obtained during the first 8 hr after feeding. The isotope ratios in lymph triglycerides were similar to that of the test meal for 4 hr after feeding and then changed to 0.70 (Fig. 1). In this study the distribution of the fatty acids in the triglycerides was determined from the isolated chylomicron fractions, rather than from whole lymph samples. There was very little deviation in the per cent of ^{14}C-fatty acid at the 2-position in the lymph compared to the meal.

Study III, W.H., Table I. Total recovery of each isotope was 35% ^{3}H and 36% ^{14}C. Despite a large volume of lymph (360 ml), the sample of the first 2 hr after feeding had little radioactivity (< 0.1% ^{3}H and < 0.02% ^{14}C). During the subsequent 7 hr, there was collected in the lymph lipids 88% of the total carbon-labeled fatty acid that was recovered; the glycerol-to-fatty acid ratio

TABLE I

Composition of thoracic duct lymph after gastric or duodenal instillation of doubly labeled glycerides

Time of meal	Lymph volume	Triglyceride	Lymph ^{14}C* / Total lymph ^{14}C × 100	$^{3}H/^{14}C$ sample‡ / $^{3}H/^{14}C$ meal	% ^{14}C in 2-position of glycerides
hr	*ml*	*g*			
Study I (B.B.) 1-:2-monostearin 30:70; total recovery ^{3}H 53% ^{14}C 57%					
					70
0–2	155	0.44	29	1.05	66
2–4	170	0.90	45	0.95	42
4–6	195	4.64	16	0.81	34
6–8	245	5.19	6	0.69	20
8–14	520	11.8	} 4	0.60	20
14–20	565	13.9		0.58	
Study II (J.K.) 1-:2-monostearin 80:20; total recovery ^{3}H 35% ^{14}C 49%					
					20
0–2	85	0.26	34	0.98	18
2–4	145	0.73	31	1.00	18
4–8	495	12.4	31	0.70	13
8–12	420	0.61	} 4	0.42	16
12–16	40	0.09		0.46	
Study III (W.H.) triolein, labeled fatty acid in 2-position; total recovery ^{3}H 35% ^{14}C 36%					
					>98
0–2	360	1.25	<1	1.12	
2–5	320	2.69	46	0.99	78
5–9	540	17.6	42	0.99	55
9–15	190	5.05	}	0.88	43
15–17	650	5.49	12	0.77	49
17–21	630	9.33		0.70	40
21–25	625			0.77	35
Study IV (F.G.) triolein, labeled fatty acid in 2-position; total recovery ^{3}H 24% ^{14}C 25%					
					91
1–2	19	0.70	35	1.01	70
2–3	60	2.79	40	0.98	74
3–4	28	1.26	5	0.94	71
4–6	136	5.06	11	0.86	63
6–8	93	1.72	}	0.88	65
8–10	50	1.17	8	0.88	61
10–12	15	0.02		0.84	
12–14	87		}	0.86	76

* Figures in this column are the percentage of recovered ^{14}C-fatty acid collected during each time interval.
‡ Glycerol-^{3}H label; ^{14}C-fatty acid label.

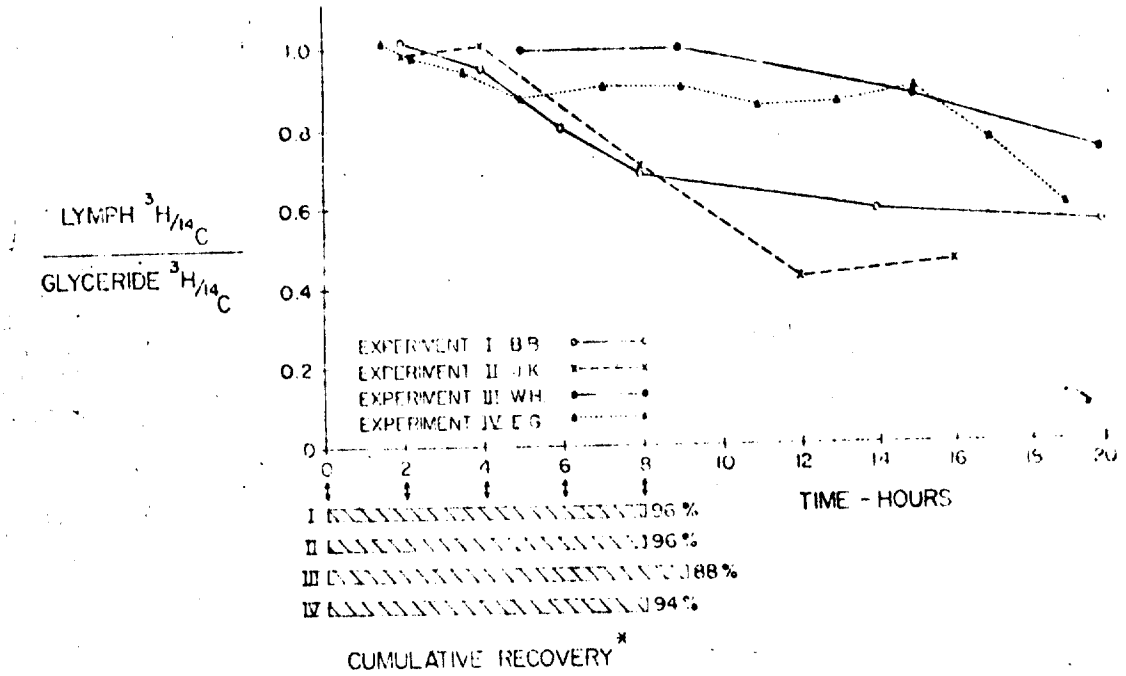

FIG. 1. COMPARISON OF ISOTOPE RATIOS IN HUMAN LYMPH LIPID AND DIETARY TRIGLYCERIDES AFTER GASTRIC OR DUODENAL ADMINISTRATION OF DOUBLY LABELED GLYCERIDES. * *Cumulative recovery* indicates the period during which the indicated per cent of the total labeled fatty acid that was recovered appeared in lymph lipid.

lymph lipids was the same as the isotope ratio of the meal (Fig. 1). 78% of the carbon label was in the 2-position for the first 3 hr after feeding and then fell for the subsequent collections.

Study IV, E.G., Table I. The total recovery of each isotope was 24% ^3H and 25% ^{14}C. The per cent of labeled fatty acid in the 2-position of the test triolein was 91. The 2nd hr sample, which contained 35% of the ^{14}C label that was recovered, had 70% of labeled fatty acid in the 2-position of the lymph triglyceride. This value for distribution of the labeled fatty acid was maintained for 4 hr, and then fell during the subsequent 8 hr.

Study V, E.G., Table II. The total recovery of the oleic acid-^{14}C was 48%. A tracer amount of labeled free oleic acid along with 20 ml of unla-

TABLE II

Composition of thoracic duct lymph after oral administration of labeled (^{14}C) oleic acid and unlabeled triolein

Study V (E.G.); total recovery ^{14}C 48%

Time	Lymph volume	Triglyceride	Lymph ^{14}C / Total lymph ^{14}C ×100	% ^{14}C in 2-position of glyceride
hr	ml	g		
0-1	48	0.25	<1	25
1-2	52	0.71	6	15
2-2.5	33	1.52	18	12
2.5-3	23	1.23	15	12
3-4	60	2.13	27	13
4-6	61	1.62	10	18
6-8	66		7	19
8-10	72		4	18
10-12	70		12	22
12-16	130			
16-20	110			
20-24	175			24

* Figures in this column are the percentage of recovered ^{14}C.

TABLE III

Absorption of radioactive glycerol into thoracic duct lymph after duodenal instillation

Study VI (S.P.) ^{14}C-glycerol; total recovery ^{14}C 4.1%

Time	Lymph volume	Lymph ^{14}C / Total lymph ^{14}C ×100
hr	*ml*	
0–0.5	20	0
0.5–1.0	45	3
1–2	130	55
2–3	80	30
3–5	155	9
5–18	360	3

* Figures in this column are the percentage of recovered ^{14}C.

beled triolein was administered to estimate the available hydroxyl sites for esterification on the absorbed monoglycerides within the intestinal cell. In this study, it is assumed that the labeled free oleic acid is almost exclusively utilized for esterification of the hydroxyl groups on the absorbed monoglycerides (from triolein), and not for synthesis via the glycerol phosphate pathway, and in addition, that there is no positional preference for oleic acid at the 1- and 3-positions of the monoglycerides. If the fatty acids were esterified to the absorbed monoglycerides in an entirely random distribution, 33% of labeled oleic acid should have been found at the 2-position of the lymph triglycerides. The data show that during the bulk of lipid absorption only 12 to 13% of labeled oleic acid was present at the 2-position, which indicates that considerably more hydrolysis and reesterification were taking place at the 1- and 3 positions than at the 2-position.

Study VI, S.P., Table III. In contrast to the greater recovery of glycerol when fed as a glyceride, only 4.1% of the radioactive glycerol was recovered from thoracic duct lymph lipid. Radioactivity appeared one-half hour after the administration of the material, and more than 88% of the total amount recovered appeared during the first 3 hr of lymph collection after instillation of the radioactive meal into the duodenum. Assay for radioactivity after fractionation by column chromatography and thin-layer chromatography revealed that the radioactivity was almost exclusively in the triglyceride fraction.

Discussion

The absorption of intact monoglycerides into the intestinal cell wall of rats was demonstrated in 1959 by Skipski, Morehouse, and Deuel (4) who used 1,3 dioleyl 2-deuterostearyl glyceride-^{14}C. Additional observations in the rat by Reiser, Bryson, Carr, and Kuiken (13) with doubly labeled monoglycerides as test meals had previously provided evidence that monoglycerides were absorbed intact into the intestinal cell wall and reesterified in the intestinal cells to triglycerides which then appeared in thoracic duct lymph. These observations were based primarily on analyses of the ratio of the two isotopes, tritum for glycerol and ^{14}C for fatty acids. Mattson and Volpenhein (5) extended these observations by analyzing in the lymph the amount of fatty acid label at each position of lymph triglycerides, after feeding monoglycerides, free fatty acids, labeled fatty acids, and triglycerides synthesized with labeled fatty acids at various positions. From these studies they concluded that hydrolysis of the dietary triglycerides in the intestinal lumen yielded 72 parts 2-monoglycerides, 6 parts 1- and 3-monoglycerides, and 22 parts free glycerol. The 2-monoglycerides, approximately three-fourths of the dietary triglycerides, entered rat intestinal cells intact, and were reesterified to triglycerides without further alteration of the fatty acid at the 2-position.

The particular role that monoglycerides have in furthering human emulsification of dietary lipids has been presented by Hofmann and Borgström (14). Analysis of intestinal lumen contents of man after fat feeding has shown a separation of glycerides into two phases: an oil phase which contains mainly diglycerides and triglycerides and some fatty acid, and a micellar phase which is a combination of bile salts, free fatty acids, and monoglycerides. From the micellar solutions, monoglycerides and free fatty acids are absorbed through the intestinal wall, leaving the bile salts within the lumen to form additional micelles.

The fate of the free fatty acids within the mucosal cell is determined by their chain length and saturation; those of less than 12 carbons are preferentially absorbed by rats directly into the portal blood, whereas longer chain fatty acids are incorporated mainly into triglycerides, and appear in lymph chylomicrons (15). Monoglycerides within epithelial cells may be esterified to di- and triglycerides or cleaved to glycerol and free fatty acids. The 2-monoglycerides are esterified especially rapidly to triglycerides, but esterification

of 1-monoglycerides lags and these are more likely to undergo hydrolysis particularly if the fatty acids are of short chain length as shown in studies in hamsters (16).

The course of drainage from intestinal lymphatics into the thoracic duct has considerable anatomic variations and is not solely into the left thoracic duct. Although the size of the cannula introduced into the duct makes it unlikely that there is much leakage around the cannula into the venous system, there are other lymphatic-venous channels that are potentially functional and may even actively serve as pathway for intestinal lymph. It has not been possible to estimate the extent of these shunts by sampling plasma and measuring the concentration of radioactivity derived from the feeding, as the turnover time for various lipid classes and the size of the vascular pool make calculations for these channels almost impossible. But blood samples taken during the course of experiments II, III, and IV revealed radioactivity in the triglyceride fraction of plasma. Recoveries of labeled fatty acids in lymph triglycerides in man range from 25 to 60%; these values are less than the 60 to 90% recoverable in rat studies with labeled fat and cannulation of the main lymph trunk in the abdomen.

The data on the absorption of glycerides obtained from these four patients demonstrate that 2-monoglyceride and its accompanying free fatty acids are the main products of triglyceride digestion and are also the principal forms in which fat is absorbed in man, just as in other animal species. It was formerly held that complete hydrolysis of triglycerides to glycerol and free fatty acids with absorption of these digestion products was a pathway for fat absorption. The results obtained in our studies demonstrate that this pathway can be of only limited importance in man. In Study VI, glycerol-^{14}C was given into the duodenum in combination with micellar solution of bile salt, monoglyceride, and fatty acid. Such a mixture was shown by Saunders and Dawson in rats (17) to give maximal incorporation of glycerol into lymph triglycerides. It should be noted that only 2.5 g of lipid was given in the micellar solution along with the radioactive glycerol. This may have reduced the amount of labeled glycerol (4.1%) recovered from the thoracic duct lymph since the supply of fatty acids may have been limited at the site of glycerol absorption. The amount recovered here is greater than that reported by Holt, who recovered only 0.7 to 1.2% of the administered glycerol-^{14}C in the 24 hr urine collection of a patient with chyluria (18). The labeled glycerol in the thoracic duct lymph was almost exclusively present as triglyceride, as determined by thin layer chromatography of the extracts of lymph lipids. The remainder of the glycerol was presumably absorbed directly into the portal blood.

Since this study in man shows that little of dietary free glycerol was incorporated into lymph lipids, then free glycerol resulting from the complete hydrolysis of triglycerides in the lumen of the intestine similarly would be incorporated only to a very limited extent into the lymph triglycerides. When labeled glycerol was fed as glycerol-glyceride, however, from 35 to 53% of the label was recovered in the lymph lipids, and absorption of glycerol therefore must have taken place as a glyceride. This is supported by the ratio of recovered ^{3}H (glycerol) and ^{14}C (fatty acid). In all four studies, during the period when most of the experimental lipid was being recovered, the ratio of the two labeled moieties in the lymph was approximately that in the diet (Fig. 1). Therefore, during the processes of digestion, absorption, and resynthesis, the glycerol and fatty acids remain associated.

The distribution of the labeled fatty acid on the triglycerides of the lymph lipids in Studies I, III, and IV shows that the main bulk of these lipids was absorbed without hydrolysis of the fatty acid located at the 2-position of the dietary glyceride. In these three studies more than half of the labeled fatty acid was still in the 2-position by the time it had reached the lymph. Only in the later time periods, after the main bulk of the dietary lipids had been absorbed, did these values drop.

Study V, in which labeled free oleic acid was given with a large amount of unlabeled triolein, supports the concept that greater hydrolysis and reesterification must take place at the 1- and 3-positions of the dietary fat, since the presence of label that could be introduced into the 2-position was only 12–13%, and not 33%, if random labeling occurred.

In Study II, when a mixture of 1- and 2-monostearin in ratio of 80:20 was fed, about 18% of the labeled fatty acid was in the 2-position of the lymph

triglyceride, a value similar to that in the dietary fat. In this patient the total recovery of ^3H (glycerol) of 85%, although less than that of the ^{14}C (fatty acid) of 49%, indicates that 1-monoglyceride was also absorbed intact. The ratio of the two isotopes in lymph lipids initially was not different from that of the dietary fat. The fate of absorbed 1-monoglycerides in the intestinal wall has been studied in animal experiments; both 1- and 2-monoglycerides are subject to hydrolysis by the intracellular monoglyceride lipase (glycerol monoester hydrolase) (19). However, 2-monoglycerides are more rapidly esterified to triglycerides (10) and incorporated into chylomicrons for delivery into the intestinal lymphatics. Under the conditions of this study, it is likely that 1-monoglycerides were also absorbed intact and esterified.

The observations reported here, together with studies on the hydrolysis of triglyceride in man, and supported by the more extensive absorption studies in animals, suggest that in man after fat ingestion, complete hydrolysis to glycerol and free fatty acid occurs to only a limited extent, especially in the first hours after a meal during which most of fat absorption occurs. The data from these studies indicate that the main route of human fat absorption into the lymph is by the direct esterification of 2-monoglycerides, derived from triglycerides, by the free fatty acids of the intestinal lumen that arise as products of pancreatic lipolysis, or by free fatty acids within the intestinal cell that are derived from monoglyceride hydrolysis and possibly from the circulating free fatty acid pool (11). Glycerol-3-phosphate derived from glucose metabolism or from phosphorylation of free glycerol can be esterified to phosphatidic acid, which then forms diglycerides (20). If large amounts of free fatty acids are present in the diet without glycerides, the glycerol portion of the lymph triglycerides would be formed from glycerol phosphate, with esterification of absorbed free fatty acids via phosphatidic acid to di- and triglycerides (21).

Calculations on the mode of triglyceride absorption in the extensive observations in animals indicate that as much as 72% of the triglyceride glycerol is absorbed as the 2-monoglyceride and reesterified to triglyceride. Our studies suggest that in man also, on the order of three-fourths of the dietary triglycerides may be absorbed as 2-monoglyceride and incorporated intact into lymph triglycerides. The monoglyceride pathway of fat absorption appears, therefore, to be the predominant route for the normal absorption of dietary triglyceride.

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EFFECTS OF OXIDIZED FATS IN THE DIET OF THE RAT

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THIS REPORT covers some preliminary results of experiments which are in progress. The objective was to determine whether oxidized fats exert a direct toxic effect on the animal. The background information on the subject is summarized in a recent review (1).

Six groups of weanling Wistar rats were placed in individual screen cages and fed the diet shown in Table 1. The experimental design is shown in Table 2. The oxidized lard and corn oil were prepared by aerating the fresh fats at 40°C. until peroxide values of 100 and 150, respectively, were attained. Groups 1, 2 and 3 received in their feed cups for *ad libitum* consumption, all components of the diet except the fat. On alternate mornings each rat was fed, by dropper, an amount of fresh lard, oxidized lard or oxidized corn oil sufficient to make up 15 per cent of its total diet. Following this dose, the feed cup was removed for a period of three hours to minimize intra-intestinal mixing of the fat with the other dietary components. The fresh fat supplement of vitamin A in corn oil was given by dropper once a week on days when no oxidized fat was fed.

TABLE 1

Experimental Diet

Casein (5 day alcohol extracted)	18
Salt mixture (McCollum #185)	4
Yeast (Fleischmann's irradiated)	5
Dextrin	56
Celluflour	2
Fat[1]	15
Fresh fat supplement[2]	

[1] Fresh lard, oxidized lard or oxidized corn oil mixed with diet or fed by dropper.
[2] 2 ml. of corn oil containing 100 I.U. of vitamin A was administered weekly to each rat.

Groups 4, 5 and 6 received in their feed cups for *ad libitum* consumption all components of the diet except the fresh fat supplement. This supplement was fed by dropper one morning each week, and the feed

cup was subsequently removed from the cage for a period of three hours. The diets were prepared fresh each week and stored at room temperature.

The growth rates of groups 5 and 6 (oxidized fat mixed in the diet) were less rapid than those of the other groups. Group 6 made the poorest growth and showed the lowest feed efficiency (grams of diet consumed per gram of weight gained) during the growing period. Diarrhea, which was observed in groups 5 and 6 during the first few weeks, was not observed in the other groups.

The rats were mated at 100-180 days of age. During the mating period a supplement of 3 mg. of α-tocopherol was given daily to each rat and an additional 10 mg. to each female on the day of littering. One male was caged with one female for a period of 20 days. If a female was not pregnant at the end of this period, she was caged with a different male from the same group for another 20 days. The litters were cut to six and equally divided between sexes insofar as possible. The young were weighed and weaned at 21 days.

TABLE 2

The Effect of Oxidized Fat on Reproduction

Group[1] Rats	Fat	Litters[2]	Rats Born	Rats Weaned	Average Weight at 21 Days (gm.)
1.	Fresh lard[3] (dropper)	9	70	29	36.1
2.	Oxidized lard[3] (dropper)	9	82	27	35.3
3.	Oxidized corn oil[3] (dropper)	7	70	26	35.8
4.	Fresh lard[4]	6	73	28	36.6
5.	Oxidized lard[4]	3	37	18	37.0
6.	Oxidized corn oil[4]	1	14	5	35.4

[1] Each group contained 12 female and 4 male rats.
[2] Twelve matings attempted for each group.
[3] Fed on alternate days in amounts sufficient to make up 15% of total diet consumed.
[4] Incorporated in the diet at a 15% level.

The reproduction record demonstrates the same trend suggested by the other data (Table 2). Groups 5 and 6 showed a low fertility, although reproduction was possible on these diets. Since the basal ration was sub-optimal for reproduction, the performance of none of the groups was equal to that of the stock colony. Cannibalism accounted for most of the losses occurring during the suckling period.

From these preliminary results it is concluded that oxidized fats fed

by dropper exerted no measurable toxic effect on the animals; however, when oxidized lard or corn oil was mixed with other components of the diet the performance of the rats, especially reproduction, was somewhat impaired. It is suggested that oxidized fat destroyed some essential component of the non-lipid portion of the diet.

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DISCUSSION

Mason: I would suggest a measurement of estrus cycles, number of matings, and number of pseudo-pregnancies as of value in these studies.

Mackenzie: Some of our results on cod liver oil and E may be of interest in connection with the papers of Dr. Quackenbush and Dr. Barnes. You may recall that we found that rabbits on a fat free diet without tocopherol developed severe muscular dystrophy and that the dystrophy could be prevented by feeding α tocopherol. However, when fresh non-rancid cod liver oil was given by mouth immediately after the tocopherol, the rabbits again became dystrophic. We felt that in order to conclusively prove that cod liver oil was increasing the requirements for tocopherol or destroying the tocopherol rather than exerting an independent effect on striated muscle, it was necessary to counteract the dystrophic action of cod liver oil by increasing the tocopherol intake. First, we attempted to do this by giving the cod liver oil and the tocopherol on alternate days. To our surprise, the rabbits developed dystrophy just as readily as when the tocopherol and cod liver oil were fed consecutively. However, when the tocopherol intake was considerably increased the dystrophic effect of cod liver oil was counteracted.[1]

Gyorgy: I believe this was the first time that evidence was presented for the *in vivo* destruction of vitamin E by cod liver oil as contrasted to destruction in the diet or in the gastrointestinal tract.

Mackenzie: Yes, I think that's right although it has received little attention in subsequent work. We also fed cod liver oil saturated with Scarlet R (unpublished data) and found that four hours later the whole mesentery was stained as well as the small intestine below the entrance of the bile duct into the lower part of the duodenum. Twenty-four hours after feeding the marked cod liver oil there was no stain to be observed at all. This was evidence that the cod liver oil was completely absorbed in less than 24 hours and hence not reacting in the intestinal tract with the vitamin E given on alternate days. One must conclude that the reaction

[1] Science, 94, 216 (1941).

was taking place in the body; that α-tocopherol is an antioxidant in the body as well as in the diet or in the lumen of the intestine, and that it is susceptible to the *in vivo* action of pro-oxidants such as those in cod liver oil. This stands in contrast to the results obtained by Dr. Quackenbush with rancid fats where E afforded no protection at all, and where feeding the fat and diet separately did give protection. It suggests (in addition to his own work) that in his experiments E was not a complicating factor but that the rancid fats were acting in some other way.

Hove: Didn't you observe that on a fat free E deficient diet, it took much longer to develop muscular dystrophy in rabbits?

Mackenzie: I don't think so. It may have taken a week or two longer to develop dystrophy on such a diet than it did on one containing cod liver oil or lard, but not months. The rabbits live longer on a low fat diet, but I think the gross and microscopic signs of muscular dystrophy appeared just about as fast as on the fat containing diets. Perhaps this is because the vitamin E depletion period in young rabbits is very short, and hence automatically limits the degree of acceleration obtainable with cod liver oil.

Mattill: That's right. The development of dystrophy is not appreciably delayed on low fat diets deficient in vitamin E as compared with the cod liver oil containing diet of Goettsch and Pappenheimer.

Mackenzie: In the early papers by the Ithaca workers evidence was presented suggesting that it was not only the fatty acid fraction of cod liver oil but also the non-saponifiable fraction that produced muscular dystrophy. As a matter of fact, we injected rabbits with pure vitamin A kindly supplied by Dr. Hickman, and found that it increased the amount of tocopherol necessary to prevent creatinuria (unpublished data). This was further evidence for the *in vivo* reaction of an antioxidant and a pro-oxidant.

Hickman: I am not surprised. This is what one would expect vitamin A to do.

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Certain Effects of Dietary Fats on the Production of Liver Tumors in Rats Fed *p*-Dimethylaminoazobenzene*

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Hydrogenated coconut oil and its component fatty acids appear to be the most striking examples of dietary lipid inhibitors reported for the carcinogen *p*-dimethylaminoazobenzene. When these substances were fed to rats as the sole sources of lipid in synthetic diets containing the azo dye very few liver tumors, if any, appeared by 6 months (3, 4). If, however, a fat such as corn oil was fed instead an incidence of 60 to 80 per cent at 6 months was obtained.

The effects of other fats on the carcinogenicity of *p*-dimethylaminoazobenzene has been the subject of two recent papers. György and his associates (1) have stated that Crisco and butter fat were co-carcinogenic as compared to lard when these fats were fed at high levels with diets low in protein, but no supporting data were offered. Opie (6) has compared diets containing various levels of Crisco, and has concluded that high levels of fat increase the rate of formation of liver tumors. The dye was fed continuously in these experiments and the animals that lived the longest (those on high fat) consumed more carcinogen than those that died early (those on low fat).

This report deals with the effects of the kind and the level of certain fats in the diet on the carcinogenicity of *p*-dimethylaminoazobenzene in rats fed a synthetic diet. Corn oil was compared with olive oil at a level of 5 per cent in the diet and corn oil, lard, and Crisco were tested at a level of 20 per cent. The series included also a diet free of added fat.

METHODS

Young albino Sprague-Dawley male rats, 130 to 170 gm. in weight, were fed 0.06 per cent of *p*-dimethylaminoazobenzene for 120 days. The animals were kept in groups of 7 and 8 in screen-bottomed cages; food and water were given *ad libitum*. After 120 days the livers were examined by laparotomy, and the animals (except Group 4) were continued on their previous diet without the carcinogen for another 60 days. At this time a final examination of the livers was made.

The diets consisted of purified casein (3) 120 gm.; salts mixture 40 gm.; one of the following quantities of fat: 50 gm. of either corn oil or olive oil, or 200 gm. of corn oil, rendered leaf lard, or Crisco (Primex); and cerelose (glucose monohydrate) to one kilogram. The diets were supplemented with the following levels of crystalline B vitamins per kilogram: 3.0 mgm. thiamin chloride, 2.0 mgm. riboflavin, 2.5 mgm. pyridoxine hydrochloride, 7.0 mgm. calcium pantothenate, and 30.0 mgm. choline chloride. Each rat received also 1 drop of halibut liver oil monthly. The carcinogen was dissolved with heat in the fat, or a solution in acetone was evaporated on the dry diet (Group 1). The rations were mixed in quantities sufficient for 1 to 2 weeks and stored at 0° C.

RESULTS

The data are presented in Table I. The general physical condition of the rats was good throughout the experiment until large tumors became evident. On the fat-free diet (Group 1) symptoms of fatty acid deficiency, consisting of a scaly dermatitis on the paws and tails, developed at about 3½ months; the dermatitis was absent in the other groups. The consumption of food, and hence of carcinogen, by this group was greater than for any other group, averaging 11 gm. per rat per day during the first 4 months. Only 2 rats in the low-fat group died before 4 months; 3 others developed hepatomas by 6 months, and 10 survived for this time without developing visible tumors. Gross cirrhosis at 4 months was absent or very mild. The final incidence of tumors found for this group, *viz.*, 23 per cent, was comparable to a previous experience with this diet (2).

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The inclusion of fat in the diet is shown by the other groups in the series. When 5 per cent of corn oil was added to the diet (Group 2) the incidence of tumors was 74 per cent, and increased to 94 per cent by 6 months. The figure is somewhat higher than our previous experience with this diet (3, 4, 5) would have led us to predict; in general an incidence of from 60 to 80 per cent at 6 months has been obtained in 8 previous trials. The substitution of olive oil for corn oil at a level of 5 per cent caused a substantial decrease in the incidence of hepatomas; to 13 per cent at 4 months and 53 per cent at 6 months (Group 3). Further, gross cirrhosis at 4 months was less severe on the olive oil diet, despite the fact that these 2 groups of rats consumed similar amounts of food; *i.e.*, 9.7 gm per rat per day.

When corn oil was fed at a level of 20 per cent a noticeable increase in toxicity and carcinogenicity occurred (Group 4). The higher level of this fat caused a reduction in food consumption to 7.3 gm. per day, and this group gained the least in weight. Large tumors were palpated before 4 months, and before any were discovered in the other groups. At 4 months the livers were severely cirrhotic, and all the rats had hepatic tumors that necessitated their sacrifice at this time. The inclusion of either of two other kinds of fat in the diet at a level of 20 per cent produced results more similar to that obtained with the lower level of corn oil. The group fed Crisco (Group 5) developed 47 and 87 per cent liver tumors at 4 and 6 months respectively, and the diet containing 20 per cent of rendered leaf lard (Group 6) produced incidences of 60 and 87 per cent. The cirrhosis present in the latter 2 groups at 4 months was comparable to that found for Group 2. The intakes of food on these 2 diets were intermediate to those observed on the high and low levels of corn oil.

TABLE I: THE EFFECTS OF THE KIND AND THE LEVEL OF CRISCO FATS ON THE CARCINOGENICITY OF *p*-DIMETHYLAMINOAZOBENZENE

Group	Diet	Average starting weight, gm	Average weight at 4 mos., gm.	Average food consumption, gm. per rat per day	Survival* at 4 mos.	Hepatomas† at 4 mos.	Hepatomas† at 6 mos.	Negative survivors at 6 mos.	Gross cirrhosis at 4 mos. none	mod erate	severe
1	Low-fat	162	221	11.0	13/15	1/13	3/13	10	11	2	0
2	5% Corn oil	161	213	9.7	15/15	11/15	14/15	1	1	8	6
3	5% Olive oil	153	236	9.7	15/15	2/15	8/15	7	13	1	1
4	20% Corn oil	162	176	7.3	15/15	15/15	-	0	0	0	15
5	20% Crisco	151	200	8.4	15/15	7/15	13/15	1	3	7	5
6	20% Lard	156	212	7.8	15/15	9/15	13/15	2	1	9	[illegible]

* Survival = Number living over number at start.
† Hepatomas = Number with hepatomas over number surviving at 4 months.

DISCUSSION

An inspection of the livers of the rats at 3 months probably would have given a more striking picture of the effect of the diet high in corn oil. The early palpation of tumors, the incidence of 100 per cent at 4 months, and the severe liver damage encountered in rats fed this diet leave no doubt as to the stimulatory action of corn oil under the conditions of these experiments. Even at a level of 5 per cent, corn oil was more stimulatory than the same level of olive oil; this comparison agrees with our previous data for these two fats (2).

The data fail to show any properties of Crisco, as compared to lard, that favor carcinogenesis, or any inhibitory effects of lard when compared to Crisco. The level of 20 per cent at which these fats were fed is close to the level of 23 per cent employed by Gyorgy and his associates (1), although these workers fed the fats in a diet containing only 6 per cent of casein. Under our conditions the high levels of lard and Crisco gave results similar to those usually obtained with 5 per cent of corn oil.

Opie (6) has studied the carcinogenicity of *p*-dimethylaminoazobenzene in diets containing either no fat of 23.8, 34.5, or 68.2 per cent of Crisco. While the amount of casein in these diets differed from one another the addition of Crisco greatly increased the ultimate carcinogenic response to the azo dye. Although the rats fed the high-fat diets lived longer and consumed more carcinogen than those given the low-fat diet, the effect of added fat is nevertheless evident in the data presented by Opie. In the present experiments the dye was fed to all groups for 4 months, and the only variation in this respect was in the different amounts of food consumed by each group. The group fed the diet low in fat received the greatest quantity of dye, yet this diet was the least toxic. The addition of only 5 per cent of corn oil caused a nearly tenfold increase in tumors at the end of the period of dye feeding, while the ingestion of 20 per cent of this fat resulted in a tumor incidence of 100 per cent at this time. Furthermore, the latter group consumed the least amount of dye.

In this paper and in previous communications we

have presented data on the effects of corn oil, olive oil, coconut oil, hydrogenated coconut oil, Crisco, and lard on the carcinogenicity of *p*-dimethylaminoazobenzene in a synthetic diet. In all these experiments rats of the same strain, sex, and age were used, and the same synthetic diet was fed throughout. It is evident that the kind as well as the level of fat in the diet is important in determining the response obtained with this agent.

SUMMARY

Six groups of 15 rats each were fed 0.06 per cent *p*-dimethylaminoazobenzene for 4 months in synthetic diets containing: no fat, 5 per cent of corn oil or olive oil, or 20 per cent of corn oil, Crisco, or lard. The dye-free diets were then fed for 2 more months. Final incidence of liver tumors of 23 per cent was produced by the low-fat diet, as compared to 94 and 53 per cent respectively when the diet contained 5 per cent of corn oil or olive oil. When 20 per cent of corn oil was fed large tumors were palpated before 4 months, and the incidence of tumors at this time was 100 per cent. When 20 per cent of Crisco or lard was given, the incidence of tumors at 6 months was 87 per cent. These 2 groups were most comparable to the group fed 5 per cent of corn oil.

The data demonstrate that in general *p*-dimethylaminoazobenzene is more carcinogenic when the diet contains fat, and further show that the incidence of hepatomas is higher in rats given a diet containing 20 per cent of corn oil instead of 5 per cent. It is therefore apparent that the level of fat in the diet, as well as the type of fat, is important for the formation of hepatic tumors by *p*-dimethylaminoazobenzene.

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Cholesterol Vehicle in Experimental Atherosclerosis

VII. Influence of Naturally Occurring Saturated Fats *

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The induction of atheromata in rabbits by cholesterol feeding usually involves administration of the sterol in an oil such as corn or cottonseed oil. This method of cholesterol administration is used because of the ease of suspension in a liquid vehicle. Although a large number of saturated natural fats have been used clinically to study the effects of dietary fats on cholesterol levels (1, 11), relatively little is known about the influences of such fats on the severity of cholesterol induced atherosclerosis in rabbits. In our early studies (3) we reported that hydrogenated vegetable oil was a more atherogenic vehicle than was corn oil, but this type of fat is not a natural fat in the strict sense of the word. Coconut oil (7, 8) and beef tallow (2) have been shown to be atherogenic for rabbits when fed in semi-synthetic diets, but not when added to commercial rabbit chow (5). Recently, *Vles* et al. (12) have shown that a diet containing coconut oil plus cholesterol is more atherogenic for rabbits than is a diet in which soybean oil is the cholesterol vehicle. Coconut oil is a naturally occurring fat, but it contains a very small percentage of fatty acids larger in size than myristic. In this report, we have compared corn oil as a cholesterol vehicle with coconut oil and with other relatively saturated fats (lard, cocoa butter and palm oil) which contain a greater proportion of longer chain saturated fatty acids.

Experimental Procedure

Male rabbits of the Dutch belted strain (average starting weight 1.7 kg) were maintained for two months on a diet consisting of rabbit chow augmented with 2% cholesterol suspended in 6% fat. At the end of the experiment the rabbits were bled by venous puncture and the serum analyzed for cholesterol. The rabbits were killed and the aortas graded visually on a 0-4 scale.

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The livers were weighed and an aliquot of each was saponified in 15% alcoholic KOH and the non-saponifiable portion extracted and assayed for cholesterol. All cholesterol determinations were carried out using the procedure described by *Mann* (10). The fatty acid composition of the fats used was determined by gas-liquid chromatography and is presented in Table I. In the experiments in which lard was used the cholesterol added to the fat was reduced by the amount of cholesterol found to be present in lard (143 mg/100 g [4]).

TABLE I

Fatty acid composition of various fats used as cholesterol vehicle

	Coconut Oil	Cocoa Butter	Palm Oil	Lard	Hydrogenated Corn Oil	Corn Oil
Iodine value	9	35	53	63	79	124
Fatty acids:						
8:0	10.0	—	—	—	—	—
10:0	6.1	—	—	—	—	—
12:0	46.7	—	0.4	—	—	—
14:0	18.5	—	1.2	1.4	—	—
16:0	9.0	23.4	45.4	25.3	10.4	11.1
16:1	—	0.4	0.4	2.6	—	0.2
18:0	2.6	40.5	6.0	12.2	2.6	0.8
18:1	5.6	32.2	36.7	45.5	79.6	29.5
18:2	0.5	1.6	8.9	10.5	7.1	56.0
18:3	—	0.2	0.2	1.4	—	0.8
20:0	—	1.2	0.5	0.4	—	0.9
% Saturated	92.9	65.1	53.5	39.3	13.0	12.8
% Monoenoic	5.6	32.6	37.1	48.1	79.6	29.7
% Polyenoic	0.5	1.8	9.1	11.9	7.1	56.8
P/S	0.005	0.03	0.17	0.30	0.55	4.44

Results and Discussion

In all, three experiments were carried out. In the first (Table II) we compared coconut oil, lard and hydrogenated corn oil with corn oil. All the more saturated fats were more atherogenic than was corn oil. In the other two experiments (Table III) we compared corn oil with coconut oil, cocoa butter and palm oil. Again, we found that of the fats used as cholesterol vehicle, corn oil was the least atherogenic. Palm oil, which has a slightly higher iodine value, than cocoa butter, was more atherogenic than cocoa butter in both experiments.

TABLE II

Influence of fat vehicle on cholesterol-induced atherosclerosis in rabbits *

Vehicle	Survival Ratio	Wt. Gain (g)	Liver Wt. (g)	Liver Cholesterol (g/100 g)	Serum Cholesterol (mg/100 ml)	Average Atheromata Arch	Thoracic
Coconut oil	12/14	105	85.1	2.56	2827	3.1	2.5
Lard	9/14	98	88.8	3.05	2245	2.7	2.0
Hydrogenated corn oil	13/14	65	73.6	2.88	1984	2.5	1.7
Corn oil	13/14	146	95.4	2.50	1908	1.6	1.3

* Diets containing 2% cholesterol and 6% fat fed for 2 months.

In the first experiment (Table II) it is evident that the differences in atherogenicity cannot be simply attributed to differences in serum cholesterol levels since there is no difference in cholesterol levels between the groups fed corn oil and hydrogenated corn oil. Serum cholesterol levels for all the groups fed cholesterol are so grossly elevated that comparison of such levels may be meaningless. The average serum cholesterol level for the normal Dutch belted rabbit is 40 mg/100 ml. Comparison of the ratio of polyunsaturated to saturated acids (P/S) could possibly be invoked to explain the differences observed in this experiment, but subsequent work (Table III) does not bear this out. Solubility of cholesterol in the vehicle does not offer an attractive explanation either, since we have shown (6) that there is little difference between solubility of cholesterol in lard and corn oil.

The experiments in which coconut oil, cocoa butter, palm oil and corn oil are compared indicate again that corn oil is the least atherogenic vehicle. Palm oil is considerably more atherogenic than is cocoa butter despite the slightly higher iodine value and P/S value. Coconut oil is consistently more atherogenic than are the other fats. The liver cholesterol levels exhibited by all groups in the second of the experiments detailed in Table III were several fold higher than those usually observed in this type of experiment and we have no explanation for this finding.

The data clearly indicate that use of the highly unsaturated fat (corn oil) as cholesterol vehicle results in less severe atheromata in rabbits. The differences in the results obtained when other fats are used as cholesterol vehicle, while roughly correlated with iodine value, cannot consistently be correlated with P/S ratio or

TABLE III

Influence of fat vehicle on cholesterol-induced atherosclerosis in rabbits *

Vehicle	Survival Ratio	Wt. Gain (g)	Liver Wt. (g)	Liver Cholesterol (g/100 g)	Serum Cholesterol (mg/100 ml)	Average Atheromata Arch	Average Atheromata Thoracic
Experiment 1:							
Coconut oil	10/10	139	86.3	2.41	1641	2.0	1.3
Cocoa butter	9/10	206	103.7	2.76	1789	1.8	1.2
Palm oil	9/10	30	104.2	2.56	1697	2.3	1.4
Corn oil	10/10	76	94.3	2.73	1676	1.4	0.9
Experiment 2:							
Coconut oil	11/12	91	102.8	8.41	1656	2.5	1.6
Cocoa butter	12/12	103	99.4	8.40	1381	1.9	1.2
Palm oil	11/12	116	104.5	8.00	1464	2.1	1.5
Corn oil	11/11	61	103.0	9.80	1463	1.4	1.2

* Diets containing 2% cholesterol and 6% fat fed for 2 months.

serum cholesterol levels. The high atherogenicity of coconut oil or trilaurin (8) when fed as part of a semisynthetic diet suggests that fatty acid chain length may play a role. In coconut oil, 90% of the fatty acids are C_{16} or smaller. In palm oil 47% of the fatty acids are C_{16} or smaller while in cocoa butter only 23% of the fatty acids are of shorter chain length than C_{16}.

The possibility arises that in addition to degree of unsaturation, the type of saturated fatty acids present in the cholesterol vehicle may influence its effect on the development and severity of cholesterol-induced atherosclerosis in rabbits. Additional evidence for the importance of the type of saturated fatty acid can be adduced from the report of *Malmros* and *Wigand* (9) that trilaurin is hypercholesteremic when fed to rabbits as 8% of a semisynthetic diet, but tripalmitin is not. Use of fats corresponding to mixtures of corn oil with saturated triglycerides of varying chain length is being contemplated in an effort to clarify this problem.

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Summary

Naturally occurring, saturated fats containing long chain fatty acids have been used as the cholesterol vehicle in the induction of atheromata in rabbits. In one experiment, coconut oil (iodine value 10) was more atherogenic than lard (iodine value 63) which was, in turn, more atherogenic than corn oil (iodine value 124). Palm oil (iodine value 53) was more atherogenic than cocoa butter (iodine value 35) and both were more atherogenic than corn oil. The data show that a highly unsaturated cholesterol vehicle is less atherogenic than a more saturated one. There is also a suggestion that among the more saturated fats, the type of fatty acid present may affect atherogenicity.

Zusammenfassung

Als Vehikel für das Cholesterin bei der experimentell erzeugten Kaninchen-Atheromatose wurden natürliche, gesättigte Fette mit langkettigen Fettsäuren verwendet. Hierbei erwies sich Kokosnußöl (Jodzahl 10) stärker atherogen wirksam als Schweineschmalz (Jodzahl 63), welches wiederum stärker atherogen war als Maisöl (Jodzahl 124). Palmöl (Jodzahl 53) zeigte eine stärkere atherogene Wirkung als Kakaobutter (Jodzahl 53), und beide erwiesen sich wiederum stärker atherogen als Maisöl. Die vorliegenden Befunde zeigen auf, daß ein hoch ungesättigter Cholesterin-Träger weniger atherogen wirkt als ein Vehikel, das aus mehr gesättigten Fettsäuren besteht. Es besteht auch Grund zur Annahme, daß unter den mehr gesättigten Fetten die Art der vorhandenen Fettsäuren für die Auslösung der Atheromatose eine Rolle spielt.

Résumé

On a provoqué une athéromatose chez le lapin avec des graisses naturelles contenant des acides de cholestérol. Dans une expérience l'huile de noix de coco (valeur de iodure 10) était plus athérogène que le lard (valeur de iodure 63) qui était à son tour plus athérogène que le beurre de cacao (valeur de iodure 35) et les deux étaient plus athérogènes que l'huile de mais. Les résultats montrent que le véhicule de cholestérol non saturé est moins athérogène que celui qui est saturé. Il est suggéré aussi que parmi les graisses saturées le type d'acide gras présent peut affecter l'athérogénèse.

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The Digestibility of Fats[1]

Langworthy-'23
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Because of the relation of fats to agriculture and their great importance in the diet, a long series of experiments on the thoroughness of digestion of animal and vegetable fats has been included in the studies of food and its uses in the home, which were made in the Office of Home Economics. The work has been carried on at intervals for several years and has been reported in bulletins of the U. S. Department of Agriculture and in other publications.[2]

The studies have included twenty-three animal fats, thirty-four vegetable fats, and six hydrogenated oils. In all cases the goods were bought in the open market or specially prepared either in the Department of Agriculture laboratories or in some other way which assured full knowledge of the process.

NOTWITHSTANDING the fact that fats and oils are an important source of energy in the diet and are two and one-fourth times as effective for this purpose as either proteins or carbohydrates, their dietetic use has received less attention at the hands of investigators than other food constituents, and is consequently less well understood.

The general assumption has been that when eaten in favorable combinations fats are well assimilated and that they do not vary enough in this respect to affect materially the amount of energy which the body receives from them. Experimental data are limited also with respect to the relation of melting point to thoroughness of digestion, particularly in the case of fats with a high melting point. This is a matter of particular interest, since increased demand for culinary and table fats tends to bring into the market kinds which hitherto have been used little, if at all.

PREPARATION OF TEST MEALS

Since it was rarely possible to find a subject who could eat fats and oils in quantity in their natural state, a method of preparing them was sought which did not submit the fats to high temperature for any considerable time, was applicable to the different kinds of fat, gave a reasonably palatable dish, and could be prepared in quantity sufficient for the entire experimental period. A method which proved satisfactory was to incorporate the fats in a sweet cornstarch pudding or blanc-mange. This was prepared by cooking in an ordinary double boiler a mixture of skim milk and sugar to which a considerable quantity of very dark caramel solution was added. When this had reached its maximum temperature (nearly 100° C.), a mixture of cornstarch and the fat under consideration was slowly added with constant stirring and the heating was continued until the starch seemed well cooked. The caramel solution gave the pudding a slightly bitter taste, but this could be readily overcome by adding a little vanilla extract when the pudding had cooled. The cornstarch pudding prepared in this way resembles a common household dessert except that it is not noticeably sweet. By this method it is possible to mask to a large extent the texture and flavor of the fats and oils. The good results obtained in this laboratory with this cornstarch mixture as a medium for supplying fat and other special foodstuffs to the diet indicate that it might prove valuable in invalid dietetics, particularly where a patient has an aversion to a large amount of fat or other foodstuff.

The cornstarch mixture was used for all the fats and oils here considered except fish and avocado fat. In these two cases the fat was not separated out. The fish meat, fat and lean together, was freed from bone and made up into fish loaf, such as had been successfully used in previous similar experiments in this laboratory. The fresh avocado pulp was fed simply with a little salt or lemon juice, as is a common custom.

Experience has shown that in such digestion experiments a simple mixed diet is more pleasing to the subject than a single food; consequently, in this work with fats the cornstarch pudding was supplemented with a very simple basal diet consisting of a commercial wheat biscuit or cracker, some fruit, and tea or coffee with a little sugar if the subject desired it.

PROCEDURE

A survey of the literature clearly shows that some variation in results can be expected in digestion experiments with different procedures, the analytical methods followed having some effect, for instance, on the determination of the quantity of fat extracted. There is also some difference of opinion regarding the desirable length of digestion periods and the methods of separating the feces; therefore the results of digestion experiments will vary within limits according to these differences in methods. However, from the extended experience, covering some twenty-five years or more, in which such problems have been studied in connection with the Department of Agriculture's food work, it is fair to conclude that where the same experimental procedure is followed, the results with various food materials are directly comparable.

The method developed and followed in this laboratory is one of several, the value of which is generally recognized. No method yet devised is absolute. Our experience, however, leads us to believe that any of these methods consistently followed throughout a series of experiments will yield re-

[1] Presented before the Division of Food Chemistry at the 64th Meeting of the American Chemical Society, Pittsburgh, Pa., September 4 to 8, 1922.

[2] "Digestibility of Some Animal Fats," *U. S. Dept. Agr., Bull.* **310**. (Beef fat, butter, lard, mutton fat.)

"Digestibility of Some Vegetable Fats," *U. S. Dept. Agr., Bull.* **505**. (Cocoa butter, coconut oil, cottonseed oil, olive oil, peanut oil, sesame oil.)

"Studies on the Digestibility of Some Animal Fats," *U. S. Dept. Agr., Bull.* **507**. (Brisket fat, chicken fat, cream, egg-yolk fat, fish fat, goose fat.)

"Digestibility of Certain Miscellaneous Animal Fats," *U. S. Dept. Agr., Bull.* **613**. (Goat's butter, hard-palate fat, horse fat, kid fat, oleo oil, oleostearin, ox-marrow fat, ox-tail fat, turtle fat.)

"Studies on the Digestibility of Some Nut Oils," *U. S. Dept. Agr., Bull.* **630**. (Almond oil, black-walnut oil, English-walnut oil, Brazil-nut oil, butternut oil, hickory-nut oil, pecan oil.)

"Digestibility of Some Seed Oils," *U. S. Dept. Agr., Bull.* **687**. (Corn oil, soy-bean oil, sunflower-seed oil, Japanese mustard-seed oil, rapeseed oil, charlock oil.)

"Digestibility of Some By-product Oils," *U. S. Dept. Agr., Bull.* **781**. (Apricot-kernel oil, cherry-kernel oil, melon-seed oil, peach-kernel oil, pumpkin-seed oil, tomato-seed oil.)

"Digestibility of Cod-Liver, Java Almond, Tea-Seed, and Watermelon-Seed Oils, Deer Fat, and Some Blended Hydrogenated Fats," *U. S. Dept. Agr., Bull.* **1033**.

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"Digestibility of Some Hydrogenated Oils," *Am. J. Physiol.*, **54** (1921), 479.

liable results and that the range of variations between individual tests with the same kinds of material will be similar whatever the method. In order to give comparable results, however, any method must be applied in essentially the same way in every case.

TABLE I—DIGESTIBILITY OF ANIMAL FATS

Fat Used in Test	Melting Point °C.	No. of Tests	Digestibility: Highest Per cent	Lowest Per cent	Av. Per cent	Corrected for Metabolic Products and Fat of Basal Ration Per cent
Butter fat	32	8	95.7	90.9	93.9	97.0
Cream	..	7	95.2	89.3	92.7	96.9
Goat's butter	..	4	94.8	92.4	93.6	98.4
Lard	35	9	95.7	88.2	93.7	97.0
Beef suet (rendered)	45	10	94.4	82.4	88.9	93.0
Beef brisket	..	7	95.9	89.1	92.8	97.4
Hard-palate fat of cattle	34	3	91.7	88.8	90.5	93.7
Ox-marrow fat	..	4	93.3	89.3	91.2	93.5
Ox-tail fat	36.8	3	95.0	89.0	93.0	96.6
Mutton suet (rend.)	50	7	88.2	72.4	80.5	88.0
Kid fat	..	3	95.2	87.7	91.3	95.3
Horse fat	..	3	95.0	92.4	93.6	93.9
Deer fat	49–54	3	75.4	71.5	73.9	81.7
Chicken fat	..	8	95.3	92.0	93.4	96.7
Goose fat	..	7	96.5	91.6	94.2	95.2
Oleo oil	..	8	92.5	87.4	90.4	96.8
Oleostearin	..	3	79.0	66.4	71.8	80.1
Egg-yolk fat	..	6	92.1	90.9	91.5	93.8
Fish fat	Room temp.	3	96.2	94.2	95.4	96.1
Turtle fat	..	4	94.5	91.8	93.3	98.6
Cod-liver oil	Room temp.	4	93.8	88.9	92.6	97.7

In the work under consideration the experimental period covers three days. The feces are marked for separation with charcoal or carmine, or both supplied with the first meal of the experimental period and the first meal after it. The feces for the entire period are dried, thoroughly mixed, and sampled, and analyzed by the standardized methods of the Association of Official Agricultural Chemists. In calculating the results, corrections are made for the small amount of fat introduced in the skim milk used in the blancmange and in the basal ration, and also for metabolic products in the feces, the factors used being based on what is believed to be ample experimental evidence. Uniformity is also observed in the choice of subjects. Generally speaking, they have been laboratory assistants or students of local medical or dental schools who had an intelligent interest in the work, which was a decided help in conducting the experiments. In a general way the subjects had more or less knowledge of the problems studied, but they were not given any definite information regarding the special foods under consideration.

TABLE II—DIGESTIBILITY OF SOME VEGETABLE FATS

Fat Used in Test	Melting Point °C.	No. of Tests	Digestibility: Highest Per cent	Lowest Per cent	Av. Per cent	Corrected for Metabolic Products and Fat of Basal Ration Per cent
Olive oil		15	95.6	91.5	94.7	97.8
Cottonseed oil		12	96.9	91.6	94.9	97.6
Corn oil		7	94.9	88.6	93.5	96.9
Sesame oil		5	95.3	92.1	93.8	98.0
Soy-bean oil		7	95.8	92.4	93.8	97.5
Sunflower-seed oil	Room temp.	4	95.2	91.9	93.8	96.5
Japanese mustard-seed oil		3	96.6	92.6	94.9	95.8
Rapeseed oil		4	96.6	93.7	95.7	98.8
Charlock (wild mustard) seed oil		4	96.3	93.7	94.6	98.9
Cocoa butter	30	11	95.5	79.1	89.6	94.9
Peanut oil		5	97.6	93.7	96.0	98.3
Coconut oil		12	96.3	88.1	93.5	97.9
Almond oil		4	96.2	86.3	93.1	97.1
Black-walnut oil		4	96.7	91.9	94.8	97.5
Brazil-nut oil		3	95.8	92.3	94.5	96.3
Butternut oil		3	94.7	85.3	88.7	95.4
English-walnut oil		3	95.6	92.5	94.4	97.6
Hickory-nut oil		4	98.4	96.5	97.2	99.3
Pecan oil		4	96.9	90.1	93.9	96.8
Apricot-kernel oil	Room temp.	4	95.4	92.3	94.4	98.4
Cherry-kernel oil		4	95.5	93.0	95.2	98.0
Peach-kernel oil		3	96.5	87.0	91.6	96.6
Watermelon-seed oil		3	94.2	88.5	90.7	94.8
Melon- (cantaloupe) seed oil		3	97.7	92.1	94.8	98.2
Pumpkin-seed oil		2	95.0	94.9	95.0	98.2
Tomato-seed oil		3	91.3	88.1	90.3	95.8
Palm-kernel oil		4	97.5	93.9	95.3	98.0
Hempseed oil		3	94.7	94.0	94.4	98.5
Poppy-seed oil		7	93.4	87.4	91.3	96.3
Cohune oil	Near 28	4	94.7	94.0	94.6	99.1
Cupuassu fat	..	4	91.0	86.3	88.8	94.1
Avocado fat	..	3	90.8	88.7	90.0	87.9
Java-almond oil	Room temp.	2	95.5	89.0	92.2	97.0
Tea-seed oil	Room temp.	1	88.2	....	....	91.2

RESULTS

In summing up the results it has seemed desirable to group the fats according to their origin, and the tables which follow show the coefficients of digestibility of animal fats in Table I, of vegetable fats in Table II, and of oils hydrogenated to a definite point in comparison with a blend obtained by mixing a softer fat with one hydrogenated to a high melting point in Table III.

TABLE III—DIGESTIBILITY OF HYDROGENATED FATS

Fat Used in Test	Melting Point °C.	No. of Tests	Digestibility: Highest Per cent	Lowest Per cent	Av. Per cent	Corrected for Metabolic Products and Fat of Basal Ration Per cent
Oils Hydrogenated to Definite Melting Points						
Hydrogenated corn oil	33.0	5	95.0	87.6	91.7	94.7
	42.0	5	93.8	89.4	91.8	95.4
	50.0	5	91.2	78.9	82.2	88.5
Hydrogenated cottonseed oil	35.0	5	96.9	89.7	93.6	96.8
	38.6	1	92.7	....	92.7	95.5
	46.0	3	93.8	91.0	92.7	94.9
Hydrogenated peanut oil	37.0	5	96.9	94.0	95.0	98.1
	39.0	3	95.4	90.2	93.3	95.9
	43.0	5	96.9	90.1	93.5	96.5
	50.0	4	90.3	86.6	88.1	92.0
	52.4	3	81.8	65.8	73.8	79.0
Hydrogenated Oils Blended to Definite Melting Points						
Blended hydrogenated corn oil	39.0	4	94.9	88.5	92.1	95.2
	49.0	3	92.4	87.0	90.4	93.3
	54.0	3	90.1	83.5	87.8	91.5
Blended hydrogenated cottonseed oil	41.3	2	92.8	91.9	92.4	96.6
	45.8	2	93.8	90.5	92.2	96.4
	47.8	4	94.0	85.4	90.7	94.2
	48.1	2	90.2	89.5	89.8	94.4
	50.0	3	85.6	76.3	82.0	87.0
Blended hydrogenated peanut oil	43.0	3	94.2	91.5	92.7	96.6
	44.2	4	97.0	91.3	94.4	97.4
	51.1	4	91.8	87.2	89.8	92.8

Consideration of the figures shows that ordinarily there are no very great differences in the digestibility of the fats studied. The animal and vegetable fats and the oils liquid at ordinary room temperature, for instance, have very much the same digestibility. It is apparent, however, that fats and oils melting above the body temperature are not quite so thoroughly assimilated, and the observed data seem to warrant the conclusion that the thoroughness of digestion is inversely proportional to the melting point. Experiments with the hydrogenated fats indicate that fats hydrogenated to a definite melting point and those made by mixing a softer fat with one hydrogenated to a much higher melting point do not differ essentially in thoroughness of digestion.

It was very noticeable in this extended series of digestion experiments and in similar series with other foods that the diet did not become monotonous and that the regularity in the diet and all that pertained to it was not irksome or disturbing. The amount of fat eaten per person per day varied within considerable limits, 50 to 60 g. being regarded as a small quantity per day, and 110 or 115 g. as a generous amount. Seldom, if ever, was any physiological disturbance noted when smaller amounts were eaten, nor was any observed in the majority of cases when the larger amounts were taken. However, it seems clear that in some cases, because of the nature of the fats themselves or because of unidentified substances accompanying them, they exercised a more or less laxative effect. For instance, this effect was observed when 140 g. of rendered beef suet were eaten per day, and was not noticeable with butter, lard, or mutton fat used in the same series of tests. In the case of olive oil and most

of the other oils used, as much as 116 to 130 g. were eaten per day with no laxative effect. In the case of cocoa butter, however, a decidedly laxative effect accompanied by slight nausea was noted, indicating that the limit of tolerance of this fat is not so high as that of the other oils. A similar effect was also noted with cupuassu fat, which comes from a tree rather closely related to the theobroma cocoa. It is also worth mentioning that a laxative effect was noted when goose fat was eaten in quantity but was not noted with a comparable amount of chicken fat. It seems not unlikely that studies undertaken to explain these observed divergencies would yield very interesting results.

Conclusion

From the investigations as a whole it seems fair to conclude that fats are wholesome, useful foodstuffs, which are well assimilated, and that for such reasons they deserve the important place they have in the diet. The fact that they are often carriers of vitamins is another reason for the esteem in which they are held.

No attempt can be made here to discuss their effect upon palatability of the diet and their important relation to the texture, flavor, and quality of the foods into which they enter, though these and other matters relating to fats are being studied in the Office of Home Economics, where it is also proposed to continue the work on the digestibility of fats when new ones or new processes of manufacture add to the list of material available for such purposes.

Acknowledgment

No account of this work carried on in the Office of Home Economics would be complete without noting the fact that much of its success is due to Mr. Arthur D. Holmes and Mr. Harry J. Deuel, who assumed direct charge of the experiments.

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Quantitative Experiments on the Production of Subcutaneous Tumors in Strain A Mice with Marginal Doses of 3, 4-Benzpyrene [1]

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INTRODUCTION

For more than a decade the production of tumors with chemical agents in experimental animals has been studied by numerous investigators. While a large amount of interesting data has been accumulated, little information is available with regard to the mechanism of carcinogenesis by such agents, as was pointed out previously (*2*).

It had been found in this laboratory that the basic fraction of creosote oil promoted tumor production with marginal doses of 3,4-benzpyrene both in skin-painting (*3, 4*) and in subcutaneous-injection experiments (*4*). This raised the question whether substances of biologic origin might be found to exert either a promoting or retarding influence on the action of carcinogens. Accordingly, the experiments described in the present paper were carried out during the years 1938–42.

In much of the published work with carcinogens, doses were selected for production of the maximum number of tumors in the shortest time. However, earlier work (*3, 4*) in this laboratory supported the viewpoint that factors which influence tumor production might be obscured when overwhelming doses of carcinogen were employed, a possibility which has been discussed by several writers (*5–8*). Consequently, experiments were carried out to ascertain the range of dose levels that would be suitable for experiments designed to reveal the influence of promoting or retarding factors. Low doses of 3,4-benzpyrene were injected subcutaneously into mice to determine the marginal range, i. e., doses large enough to induce tumors regularly in a substantial proportion of the mice but not so large as to produce tumors in all the animals.

When the present study was begun, there was little information available on the response of inbred strains of mice to the carcinogenic action of polycyclic hydrocarbons in doses below 0.2 mg. Since then Tannenbaum (*8–10*) has reported results obtained with doses of this magnitude in studies of the effect of diet on tumor genesis. Bryan and Shimkin (*11*) have attempted to place the dose-response relationship in experimental carcinogenesis with polycyclic hydrocarbons on a quantitative basis. However, there are as yet no data available on the *reproducibility* of tumor production with low doses of carcinogenic compounds under standard conditions. Blum, Grady, and Kirby Smith (*12, 13*) have studied the limits of accuracy of tumor induction with ultraviolet radiation and have considered the accuracy to be expected when experimental groups are compared, but

[1] *Presented (in part) at the Chicago meeting of the American Society of Biological Chemists, April 1941.*

it is not as yet known how applicable the findings with marginal doses of ultraviolet radiation are to those obtained with marginal doses of hydrocarbon carcinogens.

This paper records the results of 108 experiments in which 4,814 strain A mice of both sexes received, subcutaneously, doses of benzpyrene ranging from 0.2 to 0.05 mg. The purpose was to develop a standard technique which would yield results of satisfactory reliability in control groups so that a sound basis would be available for comparison with results of experiments in which promoting or retarding factors were studied. A number of solvents (lard, cetane, tributyrin, and tricaprylin) were examined for their suitability as vehicles for this purpose. Exploratory experiments were carried out with a large number of substances of biologic origin in an attempt to uncover leads on promoting or retarding agents.

However, the problem of developing a standard technique which would give results of the desired reliability in the control groups was found to assume such proportions as to necessitate far more work than had been anticipated. Accordingly, this paper deals chiefly with the question of a reproducible standard technique for tumor production by low doses of benzpyrene.

MATERIALS AND METHODS

The samples of 3, 4-benzpyrene were purified specimens with melting points between 178.2° and 179.0° C. (corrected). The lard used as vehicle was obtained from specimens of good-quality, pure leaf lard purchased on the open market. The lard was filtered at 38° for 24 to 36 hours through coarse filter paper; less than half passed through. The fraction which was liquid at 38° and which passed through the filter paper was designated "lard filtrate." The material remaining on the filter paper was designated "lard residue." Both the filtrate and the residue were employed directly; they were also further separated into fractions of different degrees of unsaturation, and into their free fatty acid, neutral fat, and unsaponifiable components. Synthetic tricaprylin was purified by various techniques including distillation at 1-mm. pressure, molecular distillation,[2] and recrystallization. The starting materials for the synthesis of the various specimens of tricaprylin, which were prepared by Hartwell (*11*), included *n*-caprylic acid (Eastman Kodak No. 665), Neo-Fat No. 7 (a commercial grade of *n*-caprylic acid), and its acid chloride. The two latter materials were obtained from Armour & Co. Commercial specimens of tributyrin (Eastman Kodak No. 726) and of cetane ($C_{16}H_{34}$), a du Pont product (boiling point 284.3°–287.3° C.; melting point 16.6°) were used without further purification. The basic fraction of creosote oil was the same preparation as that used previously (*5, 1*). A number of the other basic fractions of coal tar were obtained from the Barrett Co. The other triglycerides used were tristearin (Eastman Kodak No. 1380) with a melting point of 70°–71°, and tripalmitin (Eastman Kodak No. 1236) with a melting point of 64.5°–65.5°. They were used without further purification.

All the animals were strain A mice obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. In most cases 50 mice, equally divided between the sexes, were employed in each experiment; in a few of the earlier ones 20 to 30 animals were used. The mice were less than 6 months old at the be-

[2] *This distillation was carried out in a cyclic still manufactured by the Distillation Products, Inc., and located in the Division of Agricultural Chemical Research, U. S. Department of Agriculture, Washington, D. C.*

ginning of the experiments. Purina dog chow and tap water were freely available at all times.

The animals were given a single subcutaneous injection of 0.2 cc. of solution into the left flank. Since these oily vehicles had a high coefficient of expansion, the solutions were made up to volume at 38° C. and kept at that temperature during the injection. In the case of lard residue, which was not fluid at 38°, the experiments were performed at the slightly higher temperature required to liquefy it. Added materials were either dissolved or suspended in the solution. The animals were examined by palpation at appropriate intervals for progressively growing masses.

Tumors arising at sites other than that of injection were not included in the tabulations of induced tumors. The diagnosis of tumor was made on the basis described in a previous paper (*15*).

In the tables, the plus signs refer to the total number of mice in which tumors had been induced at the site of injection at various periods, while the minus signs refer to the negative survivors at these times; the data are expressed in percent. As in previous usage (*15*), the word "negative" denotes mice not bearing induced subcutaneous tumors. Since survival was satisfactory in most experiments, the percentages are given on the basis of the initial number of mice rather than on the effective total.

EXPERIMENTAL DATA

Lard Filtrate; Reproducibility with the Same Specimen

Table 1 summarizes the results with injections of a single dose of 0.1 or of 0.065 mg. of benzpyrene in solution in 0.2 cc. of lard filtrate. The same specimen of lard filtrate was the vehicle in all four experiments.

In the early months of these experiments, more tumors were produced with 0.1 mg. of the carcinogen than with 0.065 mg. However, by 4 months there was little difference, and by 6 months similarly high results were obtained at both dose levels. These doses gave higher yields of tumors than had been anticipated on the basis of earlier work (*1*), in which 0.1 mg. of benzpyrene dissolved in lard filtrate elicited tumors in only 30 percent of the mice at 7 months. In the present experiments, 0.1 mg. produced tumors in 84 and 94 percent, respectively, of the mice in 6 months. Even more unexpected was the high yield obtained with the lower dose of 0.065 mg.

In these two sets of experiments, in which the duplicates were carried out at different times, the same doses of benzpyrene in the same specimen of lard filtrate gave results of excellent reproducibility.

Lard Residue: Reproducibility with the Same Specimen

The fraction of lard retained on the filter paper at 38° C. was also employed as vehicle. Table 1 gives the results obtained in three experiments, performed at different times, with 0.1 mg. of benzpyrene in solution in the same specimen of lard residue. Tumor production was strikingly lower than when lard filtrate was used as the vehicle for 0.1 mg. of the carcinogen. As before, the reproducibility was satisfactory. At 9 months the percentages represent tumor production in 3, 5, and 7 mice, respectively, of an initial number of 50.

TABLE 1.—*Reproducibility of production of subcutaneous tumors with the same sample of lard as vehicle for 3,4-benzpyrene*[1] [2]

LARD FILTRATE

Experiment No.	Benzpyrene injected	Mice with tumors (+) and negative survivors (−) after various periods		
		4 months	6 months	9 months
	Milligrams	*Percent*	*Percent*	*Percent*
38a	0.1	72+ / 22−	84+ / 6−	86+ / 2−
38b	.1	88+ / 8−	94+ / 2−	94+ / 2−
43	.065	72+ / 28−	81+ / 19−	81+ / 19−
98	.065	62+ / 36−	80+ / 18−	81+ / 8−

LARD RESIDUE

39a	.1	0+ / 90−	4+ / 78−	0+ / 1−
39b	.1	0+ / 100−	4+ / 94−	0+ / 1−
100	.1	2+ / 98−	4+ / 81−	4+ / 1−
44[2]	.065	0+ / 86−	4+ / 70−	4+ / 1−

[1] Sex ratio equally divided between the sexes, except in experiment 38b.

[2] For complete data obtained in this experiment see table 1, experiment C.

VARIATION IN RESULTS WITH DIFFERENT SPECIMENS OF LARD FILTRATE

Table 2 summarizes the results of five experiments in which the same dose of benzpyrene (0.1 mg.) was injected. The factor that was varied was the specimen of lard filtrate employed as vehicle. Experiment S-1 gives, in percentages, the data obtained in a previous experiment in this laboratory (*A, table 1, experiment C, control group*). Experiments 38a and 38b, using another specimen of lard, are discussed in the section on lard filtrate. Two other experiments, not previously mentioned, were performed with two other specimens of lard.

TABLE 2.—*Variation in production of subcutaneous tumors in mice with 0.1 mg. of 3,4-benzpyrene in different specimens of lard filtrate as vehicle*

Experiment No.	Specimen of lard filtrate used	Number of mice	Mice with tumors (+) and negative survivors (−) after various periods		
			4 months	6 months	9 months
			Percent	*Percent*	*Percent*
S-1	A	20	5+ / 95−	25+ / 75−	30+ / 65−
S-2	B	20	85+ / 15−	95+ / 5−	100+ / 0−
20	C	40	30+ / 70−	55+ / 40−	60+ / 35−
38a	D	50	72+ / 22−	84+ / 6−	86+ / 2−
38b	D	50	88+ / 8−	94+ / 2−	94+ / 2−

[1] The mice were equally divided between the sexes, except in experiment S-1 in which only male mice were used (*A, table 1, experiment C, control group*).

Tumor production at the end of 9 months varied widely. Good reproducibility was obtained in the two experiments (38a and 38b) in which the same lard filtrate was employed. In all experiments but one, mice of both sexes were used; in S-1, only males had been employed. This sex difference, however, cannot account for the low tumor production in experiment S-1 as compared with the results of the other four experiments, inasmuch as it has been found (see section, Effect of Sex, p. 468) that in such experiments tumors are induced more readily in males. Since only one mouse died without tumor in 9 months in this experiment, the low tumor yield is not attributable to poor survival.

At the present time it would appear that these large differences in tumor production may be owing, in part at least, to the influence of the vehicle, which was the only factor varied intentionally in this set of experiments.

Inasmuch as such experiments require the greater part of a year for completion,

exploratory attempts were made to obtain, concurrently, some information on the possible promoting or retarding influence of biologically occurring substances. By the time the data in table 2 had been accumulated, it became apparent that the variation in the results obtained with different batches of lard filtrate, in experiments in which no additional material had been incorporated into the benzpyrene-lard solution, was greater than was considered desirable for control groups in a standardized procedure. In fact, the over-all yield of tumors and the rate of tumor production were so great in some of these experiments as to raise the question whether lard filtrate itself contained a promoting substance which varied in amount from specimen to specimen.

In subsequent sections the influence of various fractions of lard upon tumor genesis is considered, together with a brief account of the experiments in which other substances were incorporated into the benzpyrene-lard solutions. Because of the variable results obtained in the control groups when different batches of lard were employed, other materials were investigated for their suitability as vehicles.

Tricaprylin as Vehicle

Fieser (*16*) stated that a pure saturated glyceride might offer advantages over lard and suggested several, including tricaprylin, that were available commercially. He also stated that slightly yellow samples could be purified by distillation in high vacuum and that the pure glycerides were colorless, odorless, and tasteless. Accordingly, tricaprylin was employed as a vehicle for carcinogens by Shimkin and Andervont (*17*, *18*), who reported that it was excellent for this purpose. It has subsequently been used as a solvent by other workers, and it has been recommended by Shimkin (*19*) for employment in a standard technique for obtaining quantitative data in the biologic testing of carcinogens.

Tricaprylin was also used as the vehicle for marginal doses of benzpyrene in this study. Table 3 summarizes the data obtained in 12 experiments in which benzpyrene dissolved in tricaprylin was given in doses ranging from 0.05 to 0.2 mg. The four specimens of tricaprylin used were obtained from different batches of tricaprylin which were synthesized by Hartwell (*11*). Specimen A was redistilled in a molecular still in the Converse Chemical Laboratory, Harvard University, through the kindness of Professor Fieser; the melting point of this pale yellow sample was 6°–7° C. Another batch was redistilled at 1-mm. pressure, and then recrystallized without solvent; the crystals melted at 8.6°–9.2°. This specimen (B) was an almost colorless preparation. The mother liquor, which was yellow, melted at 0°–5°; it was designated specimen C. A still different batch was subjected to molecular distillation as described in the section Materials and Methods; specimen D was the fraction with the highest melting point (8.2°–9.2°). According to Dr. Hartwell *n*-caprylic acid (Eastman Kodak No. 665) was employed in the synthesis of specimen A. Neo-Fat No. 7 was used in the synthesis of specimens B and C, and the acid chloride of Neo-Fat No. 7 served as the starting material in the preparation of specimen D.

The data in table 3 show that, with any given specimen of tricaprylin as solvent, the yield of tumors induced at the injection site decreased progressively as the dose of benzpyrene was decreased from 0.2 to 0.05 mg. Similarly consistent results were obtained when all the data at different [illegible] were summarized from 15 experiments in which 7 different

specimen of tricaprylin were employed (table 4). The only apparent discrepancy

TABLE 3.—*Production of subcutaneous tumors in mice with different dose levels of 3,4-benzpyrene in the same specimen of tricaprylin as vehicle*

Experiment No.	Tricaprylin employed	Benzpyrene injected	Number of mice	Mice with tumors (+), and negative survivors (−) after various periods: 4 months	6 months	9 months	12 months
		Mg.		*Pct.*	*Pct.*	*Pct.*	*Pct.*
L 14	Specimen A	0.1	24	33+ 67−	67+ 33−	71+ 17−	71+ 0−
L 15	do	.05	23	13+ 83−	30+ 66−	47+ 44−	47+ 34−
L 23	Specimen B	.2	60	45+ 55−	68+ 30−	80+ 15−	82+ 3−
L 22	do	.1	60	12+ 87−	63+ 33−	75+ 20−	75+ 8−
L 21	do	.065	60	3+ 97−	32+ 66−	59+ 37−	53+ 10−
F 6	do	.05	48	4+ 96−	21+ 70−	38+ 40−	42+ 20−
L 10	do	.05	50	2+ 98−	24+ 73−	24+ 66−	24+ 24−
L 26	Specimen C	.2	60	23+ 73−	45+ 47−	53+ 34−	53+ 3−
L 25	do	.1	60	3+ 92−	37+ 55−	47+ 37−	49+ 13−
L 24	do	.065	60	3+ 95−	28+ 7[illegible]−	37+ 59−	43+ 28−
86	Specimen D	.1	50	28+ 66−	[illegible]+ [illegible]−	66+ 16−	
85	do	.065	50	14+ 84−	78+ 38−	60+ 28−	

[1] Mice equally divided between the sexes.

TABLE 4.—*Average rate of subcutaneous tumor production in mice with different dose levels of 3,4-benzpyrene in seven specimens of tricaprylin as vehicle*

Benzpyrene injected (in mg.)	Number of mice	Mice with induced tumors after various periods: 4 months	6 months	9 months	12 months
		Pct.	*Pct.*	*Pct.*	*Pct.*
0.2	120	34	57	67	68
0.1	194	19	56	65	66
0.08[2]	50	32	52	54	56
0.065	270	5	32	43	45
0.05	121	6	25	36	38

[1] Mice equally divided between the sexes.
[2] A single experiment was performed at this dose level.

is the relatively high value obtained with 0.08 mg. benzpyrene at 4 months. However, entirely consistent results were obtained at subsequent periods with this dose.

From these data it is evident that the dose range given in table 4 is appropriate for experiments with marginal doses of benzpyrene when tricaprylin is the vehicle. For experiments designed to ascertain promoting effects the lower end of the range, in which tumors were obtained in about a third of the mice, is considered most suitable; for studying retarding factors, the upper end of this range, in which tumors were produced in about two-thirds of the mice, is considered most suitable. In most of the experiments that were carried out with tricaprylin as vehicle, 0.065 mg. benzpyrene was employed. This dose resulted, on the average, in tumor production in about half of the animals.

The phrase "on the average" needs to be stressed. The values given in table 4 represent the arithmetic means of the values obtained in two to five experiments each, with the exception of the data for 0.08 mg. Only a single experiment was carried out at this dose level, and it is of interest that this is the only dose in which the results were not consistent at all time intervals with those obtained at other doses.

The importance of performing multiple experiments to secure reliable results is further brought out in table 5 which gives the results of the individual experiments from which the average values given in table 4 were obtained. This table contains the data secured with three specimens (E, F, G) of tricaprylin in addition to those with the four specimens recorded in table 3. Specimens E, F, and G were also synthetic preparations made by Hartwell; they [illegible] mm. pressure. The melting points are

given in table 5 except for specimen F, which was not recorded.

TABLE 5. *Variation in rate of production of subcutaneous tumors in mice with the same dose levels of 3, 4-benzpyrene in different specimens of tricaprylin (vehicle)*

Experiment No.	Tricaprylin used				Mice with tumors (T.V. and Reg.) at various periods			
	Sample	Melting point	Benzpyrene per dose	Number of mice	4 months	6 months	9 months	12 months
		°C	*Mg.*		*Pct.*	*Pct.*	*Pct.*	*Pct.*
L 23	B	8.6–9.2	0.2	97	45+ 55−	68 30	80+ 17−	82 3
L 36	C	9.0–9.0	2	60	23+ 53−	4 47	53+ 30−	53 3
L 14	A	6.0–7.0	1	4[1]	33+ 67−	67 33	71+ 17−	71 9
L 32	B	8.6–9.2	1	60	12+ 87−	63 33	75+ 20−	75 8
L 25	C	9.0–9.0	1	100	3+ 92−	35 57	47+ 37−	49 13
8	D	8.2–9.2	1	50	28+ 66	58+ 36	66+ 16−	
30	G	9.0–9.0	0.8	50	32+ 66−	52+ 46−	54+ 42−	54 34
L 21	B	8.6–9.2	0.5	60	3+ 97−	32+ 66−	50+ 37−	53+ 33−
L 24	C	9.0–9.0	0.5	60	3 95−	28+ 70	37+ 50−	43+ 28−
85	D	8.2–9.2	0.5	50	11+ 84−	58+ 30−	60+ 28−	
45	E	8.3–9.0	0.5	50	4+ 88−	35+ 53	44+ 32−	46+ 12
74	F		0.5	50	0+ 101−	10 80	22+ 30−	24+ 18
L 15	A	6.0–7.0	0.5	23	13+ 83−	0 96	47+ 44	47+ 34
E 6[2]	D	8.6–9.2	0.5	18	4+ 6−	22+ 7	38+ 19−	42 20
E 10	B	8.6–9.2	0.5	50	2+ 98−	21+ 76	21+ 69−	21 24

[1] Microscopically divided between the sexes.
[2] From unpublished work of Lloyd, Leiter, and Shear (1942).

Table 5 shows that rather wide variations in tumor production were obtained at any given dose level. These variations were great enough to result in overlapping. One possible explanation for this might [illegible] in the influence of the solvent. This is supported by the fact that when the mother liquor (specimen G) was employed as vehicle in experiments L 26 and L 25, tumor production was lower than in other experiments with 0.2 and 0.1 mg. benzpyrene in which preparations with higher melting points were used. However, with lower doses of benzpyrene, this correlation between tumor production and melting point was not evident. For example, at 9 months with 0.005 mg. benzpyrene, a higher yield of tumors (60 percent) was obtained in experiment 85 than in experiment 45 (44 percent), although the melting points of the two specimens of tricaprylin were almost identical. On the other hand, the value of 37 percent obtained in experiment L 24 was not much different from that of 44 percent in experiment 45, although the melting points (9.0° and 8.3°–9.0°) of these two specimens of tricaprylin varied widely. Similarly, with 0.05 mg. benzpyrene, the tumor incidence obtained in experiment L 15 was greater than that in experiment E 6 although the melting point of the tricaprylin used in the former experiment was about 2.5° lower. Furthermore, in experiments E 6 and E–10, in which the same specimen of tricaprylin was employed, rather wide differences in tumor production were obtained after the first 6 months.

Table 6 gives the reproducibility of the tumor incidence in experiments in which the same sample of vehicle was employed. With 0.2 mg. benzpyrene good reproducibility was obtained. With 0.05 mg., however, the reproducibility was less satisfactory. This again emphasizes the necessity of having an adequate number of experiments for the reliable determination of dose-response values even when as many as 50 mice are employed in each experi-

TABLE 6.—*Reproducibility of production of subcutaneous tumors in mice with the same sample of tricaprylin as vehicle for 3, 4-benzpyrene*

Experiment No.	Benzpyrene injected	Number of mice	Mice with tumors (+) and negative survivors (−), after various periods			
			4 months	6 months	9 months	12 months
	Mg.		*Pct.*	*Pct.*	*Pct.*	*Pct.*
L-23	0.2	60	45+ 55−	68+ 30−	80+ 15−	82+ 3−
P-4	.2	138	51+ 49−	73+ 10−	—	—
P-6	.05	48	4+ 96−	21+ 79−	38+ 40−	42+ 20−
E-10	.05	50	2+ 98−	24+ 76−	24+ 66−	24+ 24−

[1] The mice were equally divided between the sexes except in experiment P-6 in which 23 males and 85 females were used.

MISCELLANEOUS SOLVENTS AS VEHICLES

A few exploratory experiments were carried out with three other materials as vehicles for benzpyrene. The results are given in table 7.

With tributyrin (Eastman Kodak No. 726), consistent results were obtained with four doses of benzpyrene ranging from 1.0 to 0.05 mg. Tumor production at 9 months decreased progressively. Nevertheless, this solvent was considered unsatisfactory for this investigation since tributyrin, both with and without benzpyrene in solution, produced ulceration at the site of injection in strain A mice.

With cetane ($C_{16}H_{34}$, an aliphatic hydrocarbon obtained from the duPont Co., melting point 16.6° C., boiling point 284.3° 287.3°), very low tumor production was obtained even when 0.1 mg. benzpyrene was employed.

For comparison, the results obtained in experiment E-11, taken from another investigation carried out in collaboration with Dr. Lewis Engel, are also included in table 7. In this experiment, a refined, deodorized specimen of edible sesame oil [illegible] mg. benzpyrene was as great in 9 months as when 20 times that dose was given in solution in tributyrin.

TABLE 7.—*Production of subcutaneous tumors in mice with 3, 4-benzpyrene in miscellaneous substances as vehicles*

Experiment No.	Vehicle	Benzpyrene injected	Number of mice [1]	Mice with tumors (+), and negative survivors (−), after various periods		
				4 months	6 months	9 months
		Mg.		*Pct.*	*Pct.*	*Pct.*
L-10	Tributyrin	1.0	20	55+ 45−	63+ 37−	70+ 30−
	do	.5	20	40+ 60−	55+ 45−	55+ 40−
	do	.1	20	10+ 90−	20+ 80−	20+ 60−
	do	.05	20	10+ 90−	10+ 85−	10+ 75−
L-12	Cetane	.1	24	4+ 92−	4+ 67−	4+ 17−
L-13	do	.05	24	0+ 96−	0+ 83−	0+ 61−
E-11 [2]	Sesame oil	.05	50	44+ 56−	64+ 32−	68+ 28−

[1] Mice equally divided between the sexes.
[2] From unpublished experiments of Engel, Fetter, and Shear (1942).

EFFECT OF LARD FRACTIONS

When it was found that the variations obtained with different batches of lard filtrate were so great as to make advisable the selection of a more uniform substance as a standard vehicle, the question was raised whether lard contains components which facilitate or retard the carcinogenic action of benzpyrene (*20, p. 227*).

Accordingly, lard was fractionated in two ways. By crystallization from acetone according to Hilditch (*21*) four fractions were obtained which differed in degree of unsaturation. The characteristics of the fractions obtained from both the filtrate and the residue of lard are given in table 8. On comparing the iodine values with that [illegible] in which all these

fatty acid groups are unsaturated), of dioleostearin (with two of the three acid groups unsaturated), of oleodistearin (with one unsaturated fatty acid group), and of tristearin (no unsaturated fatty acid groups), it is seen that fractions I F and I R are the most unsaturated, and fraction IV R the least unsaturated. The amount of IV F obtained was insufficient for the determination of the iodine value; the degree of unsaturation of this fraction, however, was presumably similar to that of IV R.

TABLE 8.—*Characteristics of lard fractions prepared by crystallization from acetone*

Fraction	Content	Iodine value[1]
	Percent	
Lard filtrate (38)	100	[illegible]
I F	55	[illegible]
II F	9.5	[illegible]
III F	33.5	[illegible]
IV F	[illegible]	[illegible]
Lard residue (39)	100	[illegible]
I R	53	74
II R	6.5	[illegible]
III R	31	[illegible]
IV R	10	[illegible]

[1] For comparison, the iodine values of various mixed triglycerides are: triolein, 85; dioleostearin, 58; oleodistearin, 29; tristearin, 0.

In the second method of fractionation, the free fatty acids were extracted with dilute alkali, the neutral fat was saponified, and the unsaponifiable material was obtained by extraction. Table 9 contains the data on the fractions obtained by this procedure.

TABLE 9.—*Free fatty acid and unsaponifiable content of lard fractions*

Lard fraction	Content of free fatty acids	Content of unsaponifiable matter
	Percent	*Percent*
Filtrate (38)	0.[illegible]	[illegible]
Residue (39)	[illegible]	[illegible]

All the fractions were dissolved in tricaprylin (specimen D). The free fatty acid and unsaponifiable fractions were used in [illegible] lard. Three fractions were dissolved to give 10-percent solutions, by volume, in the vehicle. Fractions IV F and IV R were only slightly soluble; hence saturated solutions containing 1.8 and 1.5 percent, respectively, were employed. Benzpyrene was added to each of these solutions, and to tricaprylin alone which served as the control for the effect of the vehicle itself. The results of the experiments with these preparations are summarized in tables 10 and 11.

TABLE 10.—*Effect of fractions of lard filtrate, in tricaprylin*[1] *as vehicle, on the production of subcutaneous tumors in mice with 0.065 mg. of 3,4-benzpyrene*

Experiment No.	Lard filtrate		Mice with tumors and [illegible] after various periods		
	Fraction	Concentration in tricaprylin	1 months	[illegible]	9 months
		Per	*Per*	*Per*	*Per*
Average of L-24, 85, 87, and 74.	*Control average*	0	[illegible]	[illegible]	[illegible]
85	Control	0	[illegible]	[illegible]	60+ / 78+
87	I F	10	12+ / 82+	40+ / 46+	44+ / 22+
88	II F	10	18+ / 90+	32+ / 52+	38+ / 34+
89	III F	10	10+ / 84+	40+ / 52+	56+ / 32+
90	IV F	1.8	10+ / 94+	4+ / 76+	4+ / 68+
101	Free fatty acid	24	16+ / 84+	58+ / 42+	66+ / 28+
103	Unsaponifiable	12	16+ / 78+	44+ / 46+	58+ / 26+

[1] Specimen D.
[2] 50 mice, equally divided between the sexes, were used in each experiment.

The incidence of induced tumors obtained with the various fractions of lard filtrate is given in table 10. These solutions all contained 0.065 mg. of 3,4-benz- [illegible] cted.

TABLE 11.—*Effect of fractions of lard residue, in tricaprylin as vehicle, on the production of subcutaneous tumors in mice with 0.1 mg. of 3,4-benzpyrene*

Experiment No.	Lard residue		Mice with tumors (+) and negative surviving (−) after various periods			
	Fraction	Concentration in tricaprylin	1 month	3 months	6 months	9 months
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Average of 5 controls	*Control average*					
86	Control	0	18+ / 82−	38+ / 56−	66+ / 16−	
91	I F	10	12+ / 72−	10+ / 48−	40+ / 22−	
92	II F	10	2+ / 100−	61+ / 28−	66+ / 12−	
93	III F	10	18+ / 90−	74+ / 20−	78+ / 16−	
94	IV F	1.5	2+ / 98−	2+ / 96−	4 / 48	
104	[illegible]	.21	4+ / 96−	10+ / 82−	6+ / 16−	
105	[illegible]	.31	6+ / 88−	10+ / 26−	7+ / 11	

[1] Specific [illegible]
[2] [illegible] of [illegible] were used in experiment [illegible].

Fraction IV F exhibited a pronounced retarding effect on tumor genesis: tumors were produced in 9 months in only 4 percent of the mice. Fractions I F and II F gave a tumor yield of 44 and 58 percent, respectively, at 9 months as compared with a value of 43 percent for the average value of five control experiments with this dose of tricaprylin; these values are considered the same as the average value for control experiments.

Three fractions (III F, free fatty acid, and unsaponifiable) yielded higher results, which were close to those of the control group in this set of experiments. However, this particular control group (experiment 85) gave an unusually high tumor incidence as compared with the values obtained with 0.065 mg. in four other control experiments. On comparing the results given by the filtrate fractions with that given by the single control experiment in table 10, no evidence is apparent of a promoting action by any of the fractions. Although 0.065 mg. benzpyrene in lard filtrate (table 1) reproducibly gave definitely higher results than in tricaprylin, the source of this promoting effect was not clearly located in any of the filtrate fractions.

A possible explanation, which is susceptible to experimental test, is that one or more of the three fractions which gave the highest results may contain the material responsible for the promoting effect of lard filtrate, but that the enhancing influence was obscured by the abnormally high value given in this particular control experiment (85). Repetition of the experiments with the free fatty acid, unsaponifiable and III F fractions, together with an additional control group, might provide the desired information. If the control value should be near the average value for this dose of benzpyrene, high values yielded by these lard fractions would then take on added significance.

The results obtained with the fractions of the residue lard are given in table 11. All the solutions in this set of experiments contained 0.1 mg. benzpyrene per 0.2 cc. of solution. The same specimen of tricaprylin was employed in these experiments as in those in table 10. The control group (experiment 86) produced tumors in 9 months in 66 percent of the mice; this is the same value as the average of 65 percent obtained in four such control experiments. Incidentally, the control group at 0.065 mg. (table 10, experiment 85) gave results almost as high as those in experiment 86 (table 11) in which 0.1 mg. benzpyrene was employed. This is an additional indication that the value ob-

tained in control experiment 85 was unusually high.

As in the case of lard filtrate, the lowest result was obtained with the most highly saturated fraction (IV R). Strikingly low results were consistently obtained with those fractions of lard which consisted chiefly of saturated triglycerides.

Fractions I R and II R gave results which did not differ significantly from the control. Hence they behaved as did the analogous fractions from lard filtrate.

The three fractions of the residue, the analogues of which in the filtrate had given the highest results, all yielded more tumors than did the control. However, the differences are not considered significant except possibly in the case of III R. As in the preceding set of experiments, repetition of the work is necessary to demonstrate whether any fraction in the residue regularly exhibits promoting activity. Since this set of experiments was designed to bring out the influence of retarding factors, a dose of 0.1 mg. benzpyrene was employed, for this amount of carcinogen produces tumors in about two-thirds of the mice. In repeating the work with these fractions to reveal significant promoting influences, lower doses might be employed.

Effect of Other Saturated Triglycerides

When the pronounced difference in effect on tumor production was obtained with the filtrate and the residue of lard, it was suspected that the difference in behavior might be attributable to the difference in physical state of the vehicles for the benzpyrene. At 38°, the filtrate was a clear fluid which readily passed through filter paper. The residue was a pasty mass which was retained by the filter. It appeared to be solid fat with admixed liquid fat, for it all became liquid at a slightly higher temperature.

Tricaprylin was added to the lard residue in an amount (30 percent) sufficient to give a homogeneous, clear, liquid fat mixture. In an analogous fashion enough tristearin, a solid fat, 5 percent was added to the lard filtrate to give a pasty mass of about the consistency of the lard residue. For both preparations benzpyrene was added to give a concentration of 0.1 mg. of carcinogen per 0.2 cc. of solution at the temperature of injection. The results are summarized in table 12.

TABLE 12. *Effect of [illegible] on the production of subcutaneous tumors [illegible] benzpyrene*

Vehicle	Composition of vehicle, percent	Physical state at 38° C.	Mice with tumors at indicated times after injection period	
			4 months	7.5 months
			Percent	*Percent*
Lard residue		Paste	[illegible]	[illegible]
Do	Tricaprylin, 30 percent	Liquid	[illegible]	[illegible]
Lard filtrate		do	[illegible]	[illegible]
Do	Tristearin, 5 percent	Paste	[illegible]	[illegible]

[1] 50 mice, equally divided between the sexes, were used in each experiment.

This dose of benzpyrene in lard residue produced tumors in 8 percent of the mice in 7.5 months. Making this material liquid by addition of tricaprylin did not increase tumor production. Since 0.1 mg. benzpyrene in tricaprylin regularly produced tumors in about two-thirds of the mice (table 4), it is clear that the retarding effect of lard residue on carcinogenesis was not owing to its being a semisolid paste at 38°. A[illegible] material contained

some component which interfered with the action of benzpyrene.

Admixture of 5 percent tristearin to lard filtrate lowered the tumor production at 7.5 months from 94 to 10 percent. Inasmuch as the preceding experiment showed that the physical state of lard residue did not account for its retarding effect on carcinogenesis, the results obtained on addition of tristearin raised the question whether the retarding influence resided in certain of the saturated triglycerides. In support of this possibility are the data in table 8, second column, which show that the residue contained 20 times as much of the most highly saturated component (IV R) as did the filtrate (IV F). When these fractions were dissolved in the tricaprylin in amounts less than 2 percent they produced a striking reduction in the incidence of induced tumors (tables 10 and 11).

After exploratory experiments were conducted with tristearin and tripalmitin dissolved in tricaprylin as vehicle. The results are given in table 13. In the first pair of experiments, tricaprylin (specimen F) containing 0.065 mg. benzpyrene per 0.2 cc. of solution was employed as the control. In experiment 56, tristearin was mixed with the solution in the ratio 1:19; the amount injected was slightly greater than 0.2 cc. to allow for the increase in volume. Tumors were induced after 9 months in 84 percent of the control mice, whereas the tristearin-tricaprylin mixture gave entirely negative results.

For comparison the results with fraction IV F are also given in table 13. Here, too, a strikingly low tumor production resulted when the benzpyrene-tricaprylin solution contained only a small amount of material rich in glycerides composed of saturated fatty acids of high molecular weight.

TABLE 13.—*Effect of tristearin and tripalmitin in tricaprylin as vehicle, on the production of subcutaneous tumors in mice with 3,4-benzpyrene*

Experiment No.	Triglyceride	Concentration in tricaprylin	Benzpyrene injected	Mice bearing tumors (+) and negative survivors (−) after various periods		
				4 months	6 months	9 months
		Pct.	*Mg.*	*Pct.*	*Pct.*	*Pct.*
46	Control	0	0.065	4+ 88−	32+ 51−	44+ 32−
56	Tristearin	5	.065	0+ 96−	0+ 84−	0+ 84−
85	Control	0	.065	14+ 84−	58+ 38−	60+ 28−
90	IV F	1.8	.065	0+ 94−	4+ 70−	4+ 68−
86	Control	0	.1	28+ 66−	58+ 36−	66+ 16−
94	IV R	1.5	.1	2+ 98−	2+ 96−	4+ 78−
95	Tristearin	.4	.1	20+ 78−	44+ 56−	60+ 20−
96	Tripalmitin	1.0	.1	6+ 94−	40+ 58−	46+ 28−

[1] 50 mice, equally divided between the sexes, were used in each experiment.

Saturated solutions of tristearin and tripalmitin were then made in tricaprylin containing 0.1 mg. benzpyrene per 0.2 cc. of solution. A slight difference from the control was obtained with tristearin (experiment 95). A still lower tumor incidence was obtained with tripalmitin (experiment 96). For comparison the results given by fraction IV R are recapitulated in table 13. However, the retarding effect on carcinogenesis in experiments 95 and 96 was much less pronounced than in the other three experiments (56, 90, and 94).

It would appear from these few experiments that in order to obtain striking retardation of tumorigenesis by benzpyrene under these conditions more than 1 percent of tripalmitin or tristearin is required. As yet there is no information available on [illegible] of the [illegible] compounds

present in fractions IV F and IV R which exhibited pronounced retardation of tumor production. Since the triglycerides that occur in lard are chiefly of the mixed type, the saturated triglycerides present in these two fractions are, for the most part, presumably of the type of palmito-distearin and stearo-dipalmitin. It would be of interest to obtain data with such triglycerides and with other components which may occur in fractions IV F and IV R.

Effect of Added Substances

In parallel with the work on the reproducibility of tumor production by benzpyrene solutions in control experiments, other experiments were carried out in which various substances were added to the injected benzpyrene solution. Apart from a number of basic materials from coal tar, 11 of the 42 materials tested were compounds that occur in the animal organism. Among the substances examined were purines and amino acids.

These substances were tested in the hope of developing some leads on promoting or retarding influences concurrently with the working out of a standard technique of known reproducibility for control groups. While no conclusions have been drawn concerning the influence of these substances on tumor genesis, the results given in exploratory experiments with some of these substances are of sufficient interest to warrant further study of their action.

In commenting on the results obtained in early experiments of this nature in which lard filtrate was employed as the vehicle, it was stated (*20, p. 226*) that the production of subcutaneous tumors in mice by a borderline dose of benzpyrene was apparently being promoted by a number of nitrogen-containing ring compounds, but that further work was required to ascertain the significance of the differences. It has since been found that while differences were often obtained between test and control groups in a single set of experiments, the variability between the results in duplicate experiments with both test and control groups, when the whole set of experiments was repeated, was such as to leave uncertain the significance of the differences.

When some of the substances were tested repeatedly, however, the incidence of induced tumors was sufficiently different from the average control value, and tumors were obtained so consistently, as to raise the question whether actual retardation occurred. Viewed in this way, not as established conclusions but rather as leads for further experimental investigation, it was found that the most pronounced reduction of tumor incidence was obtained with xanthine, nucleic acid, histidine, and nicotinic acid. Low tumor incidences resulted with regularity when each of these substances was added to the injected benzpyrene in solution both in lard filtrate and in tricaprylin. In single experiments in which the substance was incorporated into the benzpyrene solution in filtered lard, pronounced retardation was given by *dl*-phenylalanine, tyramine, tyrosine, and hypoxanthine.

Incorporation of guanine in benzpyrene-lard solution yielded very few tumors in a single experiment; in benzpyrene-tricaprylin solution, however, the results were definitely lower than the average control value in only one of two experiments.

Among the anomalous results may be mentioned those with tryptophane and caffeine. When lard filtrate was the vehicle, tumor production in two experiments with tryptophane was about the same as in the controls. However, when tricaprylin was the vehicle, the results were lower in each of four experiments than in [illegible] caffeine in tricaprylin, there was evidence of retardation in both

of two experiments: in lard filtrate, however, the results did not differ much from those in the controls.

As data accumulated in this phase of the investigation, it became evident that the conclusions which might be drawn from an individual set of experiments might be erroneous even though 50 animals were employed in the control and in each of the test groups. Because of the variations in the results of experiments done in presumably the same fashion, the effect of substances placed at the site of injection of benzpyrene should be determined repeatedly before the evaluation of their role is considered reliable. The retarding effect of the substances mentioned earlier in this section are to be considered only as a suggestive finding in exploratory experiments and not as a conclusively established phenomenon.

Effect of Sex

In all experiments both sexes were employed in order to determine whether they would respond differently. Table 14 summarizes the results of 108 experiments in which 2,369 male mice and 2,443 female mice were employed. In this large number of experiments the constant factors were the strain of mouse, the carcinogen and the volume injected; the dose, the vehicle, and the presence or absence of added substances in the benzpyrene solution varied from experiment to experiment. However, in each instance both male and female mice were employed; and in 84 of the individual experiments the numbers of males and of females were equal.

At every dose level from 0.1 mg. benzpyrene to 0.05 mg., more subcutaneous tumors were induced in the males than in the females. The over-all tumor incidence for each sex was calculated by dividing the total number of mice of that sex with

TABLE 14.—*Effect of sex on the production of subcutaneous tumors in mice with 3,4-benzpyrene*

Vehicle	Experiments performed	Benzpyrene injected	Sex of mice	Number of mice	Mice with induced tumors		
	No.	*Mg.*			*No.*	*Pct.*	*Ratio*
Lard filtrate	32	0.1	Male	436	269	62	[illegible]
			Female	483	220	46	
Do.	[illegible]	.065	Male	225	191	85	[illegible]
			Female	225	134	60	
Tricaprylin	3	.2	Male	113	81	72	1.6
			Female	145	101	70	
Do.	[illegible]	.1	Male	323	233	72	[illegible]
			Female	321	152	47	
Do.	[illegible]	.08	Male	200	98	49	1.7
			Female	200	57	29	
Do.	[illegible]	.065	Male	1,010	328	32	[illegible]
			Female	1,010	188	19	
Do.	[illegible]	.05	Male	62	28	45	[illegible]
			Female	59	18	31	
Total	108		Male	2,369	1,228	52	1.4
			Female	2,443	870	36	

[1] Ratio = $\frac{\text{Percent males with tumors}}{\text{Percent females with tumors}}$.

employed. The tumor incidences for male and female mice were 52 and 36 percent, respectively. In these experiments, therefore, the incidence of induced tumors was 44 percent higher in the males than in the females.

When the tabulation was restricted to the 84 experiments in each of which equal numbers of males and females were employed, the averages for tumor incidence in the two sexes were identical with over-all values given in table 14, i. e., 52 percent for the males and 36 percent for the females. The ratio of percent incidence in males to that in females was 1.4. On further restricting the calculation to the control experiments, i. e., those in which no test compounds were added to the benzpyrene-vehicle solution and in which equal numbers of animals of both sexes were employed, the same value was obtained for the ratio of tumor incidence in the two sexes. It is therefore evident that the over-all averages represent a

phenomenon obtained regularly in the various types of experiments.

The data in table 14 include the results of a variety of experiments at each dose level of benzpyrene. Data from control experiments are summarized as well as those from experiments in which substances were added to promote or retard tumor production. For this reason deductions regarding relationships between dose of carcinogen and tumor production should not be drawn from them; these relationships have been summarized in table 4 which is restricted to the results obtained in control experiments, in which only benzpyrene was contained in the tricaprylin.

Table 15 shows the ratio of the responses of the sexes when the experiments are grouped according to the level of tumor production. The first column gives the five groups according to which the experiments were classified; for this purpose the tumor incidence in each experiment was calculated by dividing the total number of induced tumors for both sexes by the total number of mice employed. The second column gives the number of experiments performed at each level of the tumor production range. Comparative data on the incidence of induced tumors in each sex are given in the other columns. For example, there were 27 experiments in each of which the combined tumor production for both sexes had a value between 1 and 20 percent. Of the 594 male mice employed, tumors were induced at the injection site in 111, an incidence of 19 percent, whereas the analogous value for the 41 tumors induced in the 596 female mice was only 7 percent. The ratio of these values was 2.7.

The values for this ratio progressively decreased as the level of tumor production increased. The lowest ratio, 1.2, was obtained in those experiments in which tumors were produced in 80 to 100 percent of the mice.

Tumor production was not invariably greater in the males. The percent incidence and the sex ratio were therefore calculated for each of the 108 experiments. In a few instances the incidence was the same for both sexes; in a few others, it was greater in the females. The variability of the ratio from experiment to experiment in any one group was greatest in the first one, i. e., the group in which the lowest yields of tumors were obtained (0–20 percent). The few experiments in which ratios less than 1 were obtained fell chiefly in this group.

TABLE 15.—*Effect of sex on the [illegible] tumors [illegible] relation to the level of tumor production*

Level of tumor production (percent)	Experiments	Sex of mice	Number of mice	Mice with tumors		Ratio
	No.			No.	Percent	
0–20	[illegible]	Male	594	111	[illegible]	2.7
		Female	596	41	[illegible]	
21–40	[illegible]	Male	585	217	[illegible]	[illegible]
		Female	607	[illegible]	[illegible]	
41–60	[illegible]	Male	512	[illegible]	[illegible]	[illegible]
		Female	529	213	[illegible]	
61–80	[illegible]	Male	421	324	77	[illegible]
		Female	411	259	54	
81–100	11	Male	257	239	[illegible]	[illegible]
		Female	[illegible]	[illegible]	[illegible]	

[1] Ratio = $\frac{\text{Percent males with tumors}}{\text{Percent females with tumors}}$

The small number of experiments which yielded no significant difference between the sexes fell largely in the highest incidence range (81–100 percent). However, since sufficiently large numbers of mice of both sexes had been employed, a difference in response between male and female was obtained even in this range. The ratio (incidence in males: incidence in females) was only 1.2, but calculation of the statistical significance indicated that even this

low ratio represented a genuine difference in response between the sexes.

It was in the middle portion of the range, i. e., in those 67 experiments in which the tumor incidence was between 20 and 80 percent, that the sex difference was demonstrated most consistently in the individual experiments.

DISCUSSION

As stated in the introduction, the mechanisms involved in the genesis of tumors by substances like benzpyrene are almost entirely obscure. There is evidence from several lines of investigation that substances of biologic origin may promote or retard carcinogenesis by various agents. Various workers have reported on the effect of such materials, when incorporated into the diet or applied locally, on the genesis of tumors of the skin by coal tar, polycyclic hydrocarbons, or ultraviolet light; others have found that such substances influence the production of hepatic tumors when incorporated into the diet together with *p*-dimethylaminoazobenzene. More pertinent to the present experiments are the findings of those who studied the effect of various lipoid materials when used as vehicles for hydrocarbon carcinogens in the production of subcutaneous sarcomas. This literature was recently summarized by Dickens and Weil-Malherbe (*22*), who extended the findings of previous investigators on the inhibiting effect of mouse fat on sarcoma production with benzpyrene; their own preparation of mouse fat had been purified and freed from phospholipins. Injection of the same dose of benzpyrene in sesame oil or in arachis oil on the other hand, gave rise to tumors in a large proportion of the animals. They stated that it is not necessary for the lipoid material to be of homologous origin in order to obtain suppression of carcinogenic activity. They, too, were of the opinion that in such experiments the amount of carcinogen should not be too great, or any inhibitory tendency might be masked by its excessive activity.

Watson and Mellanby (*6*) painted the skin of mice with a petroleum-ether extract of mouse fat before tarring and obtained promotion of tumor genesis. In another experiment they included a large amount of butter fat in the diet before painting the skin with tar. This diet produced a greasy skin, and the mice had a higher incidence of induced skin tumors than those fed a control diet. Rusch, Baumann, and Kline (*23*) reported that local application of certain lipoids accelerated tumor production with ultraviolet light while linseed oil retarded it. Baumann and Rusch (*24*) also obtained an increased production of skin tumors with ultraviolet light in animals fed a high fat diet. In agreement with the finding of Watson and Mellanby, they noted that the skin of the mice on such a diet became greasy, and they raised the question whether a film of fat on the skin intensified the radiation effect. Tannenbaum (*8*) reported that a high fat diet gave an increased incidence of skin tumors induced with benzpyrene and of spontaneous mammary-gland carcinomas; the incidence of primary pulmonary tumors was unaffected while that of sarcomas induced by the subcutaneous injection of benzpyrene was unaffected or inhibited.

With another class of carcinogens, the azo dyes, a good deal of work has been done on the influence of various dietary factors on the genesis and development of hepatomas. It has been found that certain normal food constituents may affect the production of tumors of the liver. For example, White and Edwards (*25*) found that supplementing the diet with *l*-cystine increased the incidence of hepatic

tumor formation with *p*-dimethylaminoazobenzene from 60 to 96 percent.

When the present experiments were undertaken, it seemed likely that tumor production with polycyclic hydrocarbons might be found to be influenced by incorporating into the injected benzpyrene solution substances that occur biologically. It was found that when a few experiments were performed, together with a control group, interesting differences in tumor production were obtained. However, when both the test and control experiments were done repeatedly, with 50 mice in each group, it was found that in 2 given sets of experiments the differences from the control groups sometimes were consistently in the same direction, but in other instances they were in opposite directions. Furthermore, there were sometimes rather wide differences among control experiments in which the same dose of carcinogen was employed.

As was stated some years ago (*20, p. 227*), evidence had been obtained which indicated that variable results were produced with 0.1 mg. benzpyrene when dissolved in different batches of lard filtrate. The experiments summarized in table 2 confirm and extend these earlier findings.

Lard, of course, is a complex mixture of many substances, and it varies in composition from batch to batch. When synthetic preparations of tricaprylin were employed, it became clear that the characteristics of the specimens were important. Table 3 shows that consistently lower yields of tumor were given with specimen C as compared with specimen B. These preparations were the mother liquor and the crystals, respectively, obtained on crystallization of a sample of synthetic tricaprylin. For this reason the melting points of the specimens were recorded.

Table 4 shows the reproducibility obtained in two pairs of control experiments in which the same specimen of lard filtrate was employed throughout. In each case the duplicate experiment was performed at a later period. Nevertheless, excellent reproducibility of tumor incidence was obtained. From these data it would appear that sufficient basis existed for the belief that under standard conditions essentially similar results would be obtained. However, in experiments E-6 and E-10 (table 5), performed with the same dose of benzpyrene dissolved in the same batch of tricaprylin, the reproducibility was much poorer.

For these reasons, to obtain a sound basis for deductions with regard to promoting or retarding influences in experiments such as these may require multiple repetitions of both the control and test experiments. Of course the more striking the effect of a given factor on the incidence of induced tumors, the less necessary it is to repeat the experiment in triplicate or quadruplicate. In experiments 56, 90, and 94 (table 13) the tumor incidence was so low that it was obviously significant in view of what had already been established on the range of variability in control groups at these dose levels. Only qualitative evidence of promotion or retardation was sought; no attempt was made to estimate differences of potency in quantitative terms.

For those unfamiliar with the statistical treatment of biologic data, it may be of value to point out certain precautions to be borne in mind when use is made of dose-response curves for carcinogens, such as those of Bryan and Shimkin (*11*). While these authors stated (*11, p. 831*) that "the methods can and do visualize the accuracy and the reproducibility of the experimental results," they also called attention to the fact that "they cannot be any more accurate than the facts upon which they are based." They also found

that on the basis of available data approximately 100 animals would be required to give estimates of potency accurate to ± 0.3 of a logarithmic interval or, in arithmetic terms, accurate to $\left(\begin{matrix}+100\\-50\end{matrix}\right)$ percent. This variability appears to be small when expressed in logarithmic terms but is relatively large in terms of dosage, i. e., the response to a given dose, as expected from the curve, might also be given by half or double that dose. This may also be expressed in terms of the limits of variation in the tumor incidence at any given dose. For example, the ideal tumor incidence expected for 0.0625 mg. of 1,2,5,6-dibenzanthracene (*Fig. table 12*) is [illegible] percent but values as low as 30 or as high as [illegible] percent might be obtained by chance when [illegible] animals are used. Results in control groups accurate within this range are useful for certain purposes. However, they are of little value for the detection of modifying influences which produce in test groups changes of this magnitude in the action of the carcinogen.

In considering the limits of accuracy in tumor induction with ultraviolet radiation, Blum, Grady and Kirby-Smith (*12, p. 8*) concluded, on the basis of their extensive data:

> Strictly these findings apply only to induction of tumors by ultraviolet radiation, but it is probable that the wide variation found here is not unique in the field of cancer. If comparable latitude is found elsewhere, certain types of comparison in common use may prove unreliable.

The data reported in the present communication bear out the expectation of these tumors.

By suitably increasing the number of control experiments and the total number of mice used in such control experiments the accuracy of the dose [illegible] may be determined within narrower limits. There are a number of ways in which this may be done. In the present study the response was ascertained at 5 dose levels of benzpyrene in solution in tricaprylin. Three experiments were made with 0.05 mg. benzpyrene, 5 with 0.065 mg., 4 with 0.08 mg., 4 with 0.1 mg., and 2 with 0.2 mg. A total of 755 strain A mice was employed in these particular experiments. The mean values obtained by repeated determinations at these dose levels have far greater stability than values which might be estimated from a dose-response curve based on a small number of animals. Moreover, the data obtained in these control experiments could serve, if desired, as material for calculation of a dose-response curve of relatively high stability over the dose range studied.

It might be argued that unless a given factor exercises a striking effect on tumor genesis it is of little interest, and that it is hardly worth while to perform experiments on a large scale in order to increase the accuracy of control values if that is required to establish the significance of factors of less than overwhelming effect of carcinogenesis. However, it is conceivable that some types of so-called spontaneous tumors may arise as a consequence of the action of marginal amounts of carcinogens of biologic origin. In that event, certain factors might operate to facilitate the action of such carcinogens and others to oppose their carcinogenic action. Such speculative considerations envisage the possibility that some of these factors might be important even though their effects were less than overwhelming.

The influence of sex is a case in point. Experiments with some 4,800 strain A mice equally divided between the sexes showed that subcutaneous tumors were induced in 14 percent more males than females. In this connection the epidemi-

ologic situation in man is of interest. Many types of malignant tumors occur more frequently in men than in women. For example, leukemia, lymphosarcoma, Hodgkin's disease, gastric carcinoma, and primary pulmonary carcinoma in human beings have a higher incidence in males than in females (*26, 27*). One of the hypotheses which have been offered in explanation of the difference in sex distribution of these nepolasms is the greater exposure of men to certain postulated environmental conditions that facilitate the genesis of tumors of these types. However, the findings of the present study, in which both sexes were exposed to the same environmental influences, raise the question whether sex physiology plays a role in the higher incidence of some of these types of tumors in men.

Fieser (*10*) summarized the earlier work, particularly of Andervont, of Boyland and Warren, and of Dunning, Curtis, and Bullock, with reference to the influence of sex on the genesis of subcutaneous tumors by polycyclic hydrocarbons. He stated (*10, p. 50*):

> It may be concluded that with a large majority of the species and strains currently used in studies of induced carcinogenesis sex plays no significant role in determining the response to tumor-producing hydrocarbons.

The only apparent exception was noted in the C[illegible] Black strain; in these mice the latent period was shorter in the males than in the females in two experiments. However, the tumor incidence in one of these two experiments was significantly lower in the males.

There may be several reasons why the sex difference, found so consistently in our experiments, was not seen in the literature summarized by Fieser. The most obvious one, however, would appear to be the relatively large doses of carcinogen employed: the lowest dose was 0.8 mg. of [illegible]. In most of the experiments summarized by Fieser, tumors were produced in a high proportion of the animals. In our experiments the sex difference was least noticeable when tumors were produced in more than 80 percent of the mice.

In the Japanese work summarized by Cook and Kennaway (*28, p. 539*) administration of "androstin" (a "male hormone") together with 4′-amino-2,3′-azotoluene resulted in the production of hepatomas in more male rats than in other experiments in which the azo compound was given alone or together with cholesterol; none of the females in any of the three groups showed hepatomas. These results were in agreement with the earlier ones obtained by Tomita in which a male-hormone preparation had a considerable accelerating action upon the development of hepatomas by this azo compound in male but not in female rats. The authors, Amano and Tomita, were inclined to correlate their results with the greater prevalence of cancer of the liver in men than in women in Japan. Cook and Kennaway also summarized the work of Ito who gave this same azo compound to normal and castrated female rats; castration appeared to hasten the development of hepatomas.

In the section of their review entitled "Oestrogens in Relation to Tumours," Cook and Kennaway brought together a large amount of interesting data obtained by various workers. For example, Lacassagne observed sarcomas and epitheliomas, following injection of estrogen, in strains of mice in which they were known not to have occurred spontaneously for several years, from which he concluded that the estrogenic hormones played a part in their production. For summaries of the contradictory literature on the effect of the gonads and sex hormones on carcinogenesis, the reader is referred to the reviews of Cook and Kennaway (*28*) and of Stewart (*2[illegible]*) [illegible] found that

tion of mice of both sexes of the C3H strain, followed by subcutaneous injection of 0.8 mg. of 1,2,5,6-dibenzanthracene in lard solution, resulted in more rapid production of tumors in the castrates as compared with the intact control mice of each sex. This effect was evident up to the sixteenth week; subsequently, tumor production in the intact groups was sufficiently great so that these differences were no longer evident by the time the experiments were terminated. Stewart stated (*29, p. 1056*): "Whether the differences might have been brought out more strikingly by employing a weaker dose of the carcinogen it is impossible to state at present."

It is by now well-established that in C3H mice spontaneous hepatomas occur much more frequently in males than in females. The earlier work on the relation of sex physiology to hepatomas induced by azo compounds has been summarized in the papers mentioned previously in the discussion. More recently, Andervont and Edwards (*30, 31*) have found that the females of all strains receiving 4-aminoazotoluene are far more susceptible than the males to induced macroscopic hepatic lesions and hepatomas. It therefore appears that the chemical mechanisms involved in the reactions of compounds which have a selective action on the liver are affected by sex physiology in a direction opposite to that observed in the effect of benzpyrene upon subcutaneous tissue. It is of interest in this connection to recall that direct application of hydrocarbon carcinogens to the liver showed this organ to be refractory to the carcinogenic action of these agents (*32*).

Although the processes involved in the successful growth of implants of transplantable tumors are different from the genesis of tumors by chemical carcinogens, it is worthy of note that Gross (*33, 34*) reported that sex plays a part in the percentage of takes on transplantation of mouse sarcoma 37. Male mice were found to be more susceptible than females to intradermal implantation of this tumor. Mice of both sexes, in which the tumors produced by this technique had regressed spontaneously, were reinoculated intradermally or intraperitoneally with the same tumor; most of the females were found to be resistant to reinoculation, whereas one-fourth of the males developed tumors.

No conclusions have as yet been drawn with regard to the effect, on tumor production, of the substances added to the benzpyrene solutions. The results of the preliminary experiments indicate, however, that it may be worth while to investigate further the effect of certain of these substances, particularly the purines.

Purines have been found (*35*) to have the property of making benzpyrene soluble in an aqueous medium. Among the purines tested were theobromine, theophylline, xanthine, and caffeine. A 3-percent aqueous solution of caffeine was found to bring benzpyrene into solution to a concentration of 20 mg. per 100 cc. In view of the results obtained in our exploratory experiments with xanthine, caffeine, and other purines, it would seem that further work with these substances might provide interesting findings.

Twort and Twort (*36*), in skin-painting experiments with dibenzanthracene and with benzpyrene, employed a number of fatty acids as vehicles, viz. oleic acid, butyric acid, and mixtures of oleic and stearic acids and of oleic and butyric acids. The results indicated that butyric acid diminished the potency of the carcinogen while stearic acid appeared to be without effect. They stated: (*36, p. 83*): "Perhaps the most interesting of the findings which have arisen from these experi-

ments is the confirmation of the sensitising effect of oleic acid." They also reported (*36, p. 85*): "Cells rendered abnormal by a few applications of benzpyrene quickly pass into the irreversibly cancerous phase when stimulated by oleic acid. Liquid paraffin, lanolin, and some other agents apparently have no such action." Berenblum (*37*) found that croton oil caused a noticeable augmentation of carcinogenesis when applied to the skin of mice in conjunction with a very dilute solution of benzpyrene in acetone; croton resin, a constituent of croton oil, exhibited a similar cocarcinogenic action to an even greater degree.

In the present work the most outstanding finding, apart from the influence of sex, was the effect of lard on tumor genesis by benzpyrene. In comparison with tricaprylin as the control vehicle, lard residue produced a striking retardation of tumor production whereas lard filtrate promoted it. Further fractionation indicated which components may be responsible for the promoting action and demonstrated that the retarding influence resides in the glyceride fraction richest in saturated fatty acids of high molecular weight.

SUMMARY

Quantitative experiments on tumor production were performed with about 3,000 strain A mice injected with marginal doses of 3,4-benzpyrene, i. e., with doses large enough to produce tumors in an appreciable percentage of the mice but below the dosage required for production of tumors in all the animals. A single injection of benzpyrene was given subcutaneously in the dose range of 0.05 to 0.2 mg. of the carcinogen. Various lipoid materials were used as vehicles for the benzpyrene. In most of the 108 experiments, 50 mice, equally divided between the sexes, were used.

Lard filtrate was the vehicle in five experiments with 0.1 mg. benzpyrene. Tumor production varied widely in these experiments. At 6 months, for example, the incidence of induced tumors ranged from 25 to 95 percent. These variations were so large as to militate against the use of this material as a vehicle in certain types of quantitative studies.

The variations were apparently a consequence of the different proportions of retarding and promoting substances in the various specimens of lard filtrate employed. Lard residue exercised a striking retardation of tumor genesis by benzpyrene. On the other hand, when lard filtrate was the vehicle, rapid tumor production in a high proportion of the animals was usually obtained. Fractionation of both the filtrate and residue of lard by various methods showed the retarding influence to reside in the glycerides which were richest in saturated fatty acids of high molecular weight.

Several other materials were tested for suitability as vehicles in quantitative experiments. The most suitable of these was found to be tricaprylin. Synthetic preparations of this triglyceride were found to vary in their effect on carcinogenesis; in some instances this appeared to be correlated with the melting points of the specimens.

Duplicate experiments, carried out at different times, sometimes gave results of satisfactory reproducibility. In other instances, however, repetition of experiments gave results which varied more than was considered desirable for certain types of comparison. Consequently, in ascertaining the dose-response relationships in the range in which tumors were produced in one-third to two-thirds of the animals, the experiments were performed repeatedly. In these standard reference experiments in which the vehicle was 0.2

cc. of tricaprylin, the following average values were obtained for tumor production in 12 months: In three experiments in which the dose of benzpyrene was 0.05 mg., the tumor incidence was 38 percent; in five experiments with a 0.065-mg. dose of benzpyrene, the tumor incidence was 45 percent; and in four experiments using 0.1-mg. doses of benzpyrene, the tumor incidence was 65 percent.

Exploratory experiments were carried out with a large number of substances of biologic origin incorporated into the benzpyrene solution prior to injection. Indications of a retarding effect on tumor production were obtained with some of these substances.

The incidence of induced subcutaneous tumors was about 50 percent higher in the male mice than in the female.

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REGULATION OF CHOLESTEROL SYNTHESIS IN THE LIVER: THE INFLUENCE OF DIETARY FATS*

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A variety of factors have been shown to control cholesterol synthesis in the liver. The nutritional state of the animal has a decided influence, and it has been demonstrated that fasting for a matter of 24 to 72 hours depresses, to a considerable extent, the capacity of the rat liver to incorporate acetate carbon into cholesterol (1). Restriction of caloric intake has a similar effect (1). Fat, carbohydrate, and protein appear to be equally effective in restoring hepatic cholesterogenesis in the fasted rat (1). The liver's capacity for cholesterol synthesis is also regulated by cholesterol consumption. For example, it was observed by Tomkins *et al.* (2) that the liver of a rat fed 8 days a diet entirely devoid of cholesterol has a much greater capacity for cholesterogenesis than does the liver of a rat fed a 0.5 per cent cholesterol diet. A homeostatic regulation for hepatic cholesterol synthesis has also been reported by Gould *et al.* (3), Frantz *et al.* (4), Cox *et al.* (5), and Swell *et al.* (6). A third type of control is mediated by endocrines. Thus, the incorporation of added acetate into cholesterol is much greater in liver slices prepared from diabetic rats than in those prepared from normal rats (7, 8). Although hepatic cholesterogenesis is reduced in the liver of the hypophysectomized rat fed an adequate stock diet (9, 10), it can be made to proceed at normal rates by the feeding of a synthetic diet high in available carbohydrate (11).

Many recent reports have emphasized an influence of dietary fats upon cholesterol metabolism (12, 13), particularly upon the levels of serum cholesterol in man (14, 15). The mechanism of this action of dietary fat is not understood. Since the liver is a principal site for the formation of plasma cholesterol (16), we have studied here the effects of fats, animal as well as

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vegetable, upon the liver's capacity for synthesizing cholesterol from acetate carbon.

EXPERIMENTAL

Treatment of Animals.—Long-Evans rats were fed synthetic diets for 3 days before they were sacrificed. The control diet consisted of 50 per cent glucose, 37 per cent vitamin free casein (Nutritional Biochemical Corp.), 4.8 per cent cellaflour, 6 per cent salt mixture (37), 2 per cent defatted liver (VioBin), and an adequate vitamin B mixture (18). Changes in the fat content of other diets were made at the expense of cellaflour or cellaflour and casein.

Preparation of Tissues and Incubation Procedure.—Blood was withdrawn from the rats by cardiac puncture just before they were sacrificed. Their livers were removed and immersed in a chilled Krebs-Henseleit bicarbonate buffer (19). Slices approximately 0.5 mm. thick were prepared with a mechanical tissue slicer (20), and 500 ± 5 mg. portions were placed in the main compartment of a 50 ml. incubation flask containing 5.0 ml. of the bicarbonate buffer (pH 7.3–7.4) to which acetate-1-C^{14} as the sodium salt had been added. The flask was gassed with a mixture of 95 per cent O_2 and 5 per cent CO_2, and closed with a self sealing rubber cap. The tissues were incubated, with shaking, at 37.5° for 3 hours.

Analytical Procedures.—The methods used for determining $C^{14}O_2$ in respired gas and C^{14} cholesterol of liver were described earlier (11). The procedure for measuring plasma glucose and liver glycogen have also been described elsewhere (11). Plasma and liver fatty acids were determined by the method of Bragdon (21), and plasma and liver cholesterol by a modification of the method of Zak *et al.* (22).

RESULTS

1. *Experiments with Diets Containing 15 Per Cent of Various Fats.*—

The first group of rats (1 to 8, Table I) was fed, for 3 days before they were sacrificed, a diet devoid of fat. When 500 mg. of slices prepared from their livers was incubated with acetate-C^{14}, approximately 1 per cent of the C^{14} was incorporated into cholesterol. The other rats (9 to 24) were fed, for the same 3 days, diets to which had been added 15 per cent of either corn oil (Mazola), Wesson oil, hydrogenated vegetable oil (Snowdrift), or lard. The calorific value per gram of these fat-containing diets was made the same as that of the fat-free diet. The average daily food intake during the 3 days of observation was about the same for each group of rats studied.

The livers of all rats that had been fed the fat-containing diets showed an augmented capacity for converting acetate carbon to cholesterol.

Under the conditions of the short term feeding, the addition of fat to the diet did not affect the levels of glucose, fatty acids, and cholesterol of plasma nor the glycogen, fatty acids, and cholesterol content of the liver (Table I).

2. *The Question of Protein in the Diet.*—In the construction of the diets used in the preceding experiments, isocaloric values per gram of diet were maintained by reducing the casein content from 37 per cent in the fat-free diet to 15 per cent in the diets to which fat had been added. It therefore became necessary to determine whether protein, *per se*, can affect hepatic cholesterogenesis. To test this point, rats were fed, for 3 days before they were sacrificed, fat-free diets containing 50 per cent glucose and either 37 or 15 per cent protein. The results, recorded in Table II, show that a doubling in

TABLE I

Effect of Dietary Fat upon Hepatic Cholesterogenesis

Duplicate 800 ± 5 mg. portions of liver slices were incubated at 37° for 3 hrs. in 5.0 ml. of bicarbonate buffer containing 2 mM acetate-C^{14}. Average values are reported.

Rat		Diet			Average daily food intake	Plasma			Liver					Percent of added acetate-C^{14} recovered as:	
No.	Weight	Glucose	Fat	Casein		Glucose	Fatty acids	Cholesterol	Weight	Glycogen	Fatty acids	Cholesterol		CO_2	Cholesterol
	gm.	per cent	per cent	per cent	gm.	mg. per cent	mg. per cent	mg. per cent	gm. (wet)	per cent wet wt.	per cent wet wt.	per cent wet wt.			
1	284	50	None	37	12	81	282	41	4.05	3.72	2.75	0.35		32	0.69
2	260	"	"	"	15	97	236	37	4.59	4.[illegible]	2.44	0.22		35	1.09
3	295	"	"	"	14	92	231	35	4.82	5.53	2.52	0.25		29	0.82
4	285	"	"	"	16	85	212	38	4.35	4.95	2.35	0.25		33	0.98
5	220	"	"	"	13				3.04	5.89	2.07	0.28		33	1.24
6	240	"	"	"	14				4.21	5.80	2.03	0.29		28	1.19
7	250	"	"	"	14				4.56	6.70	2.22	0.27		30	1.35
8	240	"	"	"	16				4.60	4.72	2.04	0.27		27	1.16
Averages						88	240	38	4.34	5.36	2.30	0.28		31	1.09
9	280	50	15 CO*	15	12	81	255	42	4.42	3.16	3.06	0.30		33	2.36
10	275	"	"	"	13	78	268	37	3.58	3.[illegible]	3.15	0.28		32	2.86
11	320	"	"	"	14	87	248	58	3.40	3.11	3.10	0.27		32	1.90
12	285	"	"	"	8	67	285	46	3.55	4.08	3.12	0.27		32	2.69
Averages						78	261	46	3.69	3.72	3.10	0.28		32	2.45
13	250	50	15 L*	15	12	79	240	41	3.69	3.18	2.62	0.21		33	3.65
14	240	"	"	"	9	84	261	38	4.19	4.49	2.98	0.30		34	3.10
15	310	"	"	"	13	88	256	45	3.62	4.02	2.81	0.25		37	2.55
16	310	"	"	"	13	94	219	51	3.95	4.15	2.78	0.28		31	5.45
Averages						86	244	44	3.82	4.13	2.85	0.27		34	3.54
17	270	50	15 VO*	15	14				3.71	3.95	2.92	0.29		33	3.53
18	280	"	"	"	13				4.61	5.75	2.61	0.29		31	3.24
19	230	"	"	"	11				3.56	6.12	2.53	0.32		32	3.62
20	230	"	"	"	13				4.25	5.27	2.96	0.30		33	3.58
Averages									4.03	5.26	2.75	0.30		32	3.51
21	250	50	15 HVO*	15					4.09	4.85	2.65	0.31		33	4.02
22	310	"	"	"					4.04	4.82	2.73	0.32		26	3.46
23	290	"	"	"					4.41	4.20	3.00	0.32		30	3.12
24	240	"	"	"					4.20	4.70	2.65	0.33		28	2.72
Averages									4.18	4.72	2.76	0.33		29	3.33

* CO, corn oil (Mazola); L, lard; VO, vegetable oil (Wesson); HVO, hydrogenated vegetable oil (Snowdrift).

the protein intake by the rat had no effect on the liver's capacity for synthesizing cholesterol from acetate. It would appear that the observed increase in hepatic cholesterogenesis shown in Table I resulted from ingested fat.

3. *The Question of Insulin Secretion.*—It has been reported that the feeding of fat reduces the insulin content of the rat pancreas (23). This observation, in conjunction with an earlier finding that the liver of the diabetic rat has an enhanced capacity for converting acetate carbon to cholesterol (7), led us

TABLE II

The Effect of Dietary Protein upon Hepatic Cholesterogenesis

For experimental details see Table I.

Rat		Diet*		Average daily food intake	Liver				Per cent of added acetate-C^{14} recovered as	
No.	Weight	Glucose	Casein		Weight	Glycogen	Fatty acids	Cholesterol	CO_2	Cholesterol
	gm.	*per cent*	*per cent*	*gm.*	*gm. /100 gm.*	*per cent wet wt.*	*per cent wet wt.*	*per cent wet wt.*		
24	230	50	37	12	5.21	4.16	3.12	0.28	32	1.63
26	250	"	"	16	4.63	4.40	2.96	0.32	28	1.58
27	240	"	"	12	4.74	5.06	2.78	0.26	31	1.94
28	235	"	"	13	4.80	4.16	3.05	0.27	26	1.82
29	265	"	"	9	5.26	3.60	2.69	0.28	33	1.18
Averages					4.90	4.28	2.98	0.28	30	1.63
30	230	50	15	15	4.30	4.70	3.28	0.28	30	1.25
31	255	"	"	9	4.62	4.20	2.48	0.30	26	1.16
32	295	"	"	12	4.90	5.10	2.76	0.33	28	1.35
33	205	"	"	14	5.20	4.70	2.90	0.27	39	1.89
34	195	"	"	13	4.70	4.05	2.88	0.29	30	1.10
Averages					4.74	4.55	2.86	0.29	31	1.35

* The diets contained no added fat.

to consider the possibility that the effect of ingested fat on hepatic cholesterogenesis in the normal rat may reflect a decreased production of insulin. The results presented in Table III, however, demonstrate that the administration of extra insulin to normal rats fed the fat-containing diets did not reduce hepatic cholesterogenesis to the levels observed in normal rats fed the fat-free diets.

4. *Studies with Intact Rats.*—The data reported so far were obtained from experiments with liver slices. The feeding of fat also increased the recoveries of C^{14}-cholesterol in the livers of normal rats injected with acetate-1-C^{14}.

TABLE III

Effect of Insulin on Hepatic Cholesterogenesis in Normal Rats Fed Fat-Free and Fat-Containing Diets

For experimental details see Table I.

Rat		Diet			Insulin	Average daily food intake	Liver				Percent of administered C¹⁴ recovered as:		Average blood glucose
No.	Weight	Glucose	Corn oil	Casein			Weight	Glycogen	Fatty acids	Cholesterol	Glycogen	Cholesterol	
	gm.	*per cent*	*per cent*	*per cent*		*gm.*	*gm./100 gm.*	*per cent wet wt.*	*per cent wet wt.*	*per cent wet wt.*			*mg. per cent*
35	220	50	0	37	None	12	3.90	4.36	2.71	0.2[illegible]	31	1.58	86
36	190	"	"	"	"	14	4.10	3.22	2.58	0.33	34	1.99	
37	205	"	"	"	"	10	3.95	4.66	2.74	0.26	33	1.76	
38	230	"	"	"	"	12	3.88	3.50	2.69	0.26	30	1.86	
Averages						12	3.90	3.93	2.68	0.28	32	1.80	
39	180	50	0	37	Yes*	14	4.21	2.08	2.50	0.30	32	1.70	41
40	195	"	"	"	"	16	3.56	5.70	3.22	0.29	32	1.36	
41	245	"	"	"	"	20	3.42	5.40	2.62	0.26	26	1.29	
42	220	"	"	"	"	16	3.95	5.92	2.68	0.28	30	1.96	
Averages						16	3.78	4.78	2.75	0.28	30	1.58	
43	210	50	15	15	None	8	4.21	3.40	4.22	0.32	36	4.25	91
44	210	"	"	"	"	13	4.02	4.72	3.16	0.30	34	3.06	
45	185	"	"	"	"	12	3.75	3.36	4.05	0.37	32	4.55	
46	220	"	"	"	"	12	3.90	4.96	3.72	0.28	25	4.15	
Averages						11	3.99	4.11	3.78	0.31	32	4.00	
47	19[illegible]	50	15	15	Yes*	16	3.82	3.78	2.92	0.30	32	4.15	36
48	190	"	"	"	"	20	3.68	4.56	3.18	0.23	34	3.94	
49	215	"	"	"	"	13	4.15	4.82	3.78	0.28	33	2.96	
50	205	"	"	"	"	18	3.98	3.97	4.02	0.29	30	3.55	
Averages						18	3.90	4.27	3.47	0.29	32	3.65	

* Protamine zinc insulin 4 u. per kg. body weight. It was given in 2 equal doses each day for 3 days before the rat was sacrificed.

Rats weighing 225 to 250 gm. were fed, for 3 days, isocaloric diets containing either no fat or 15 per cent corn oil. At the end of that time each rat was injected intraperitoneally with 0.5 ml. of 0.9 per cent sodium chloride solution containing the sodium salt of acetate-1-C^{14}. Each 0.5 ml. of this solution contained 3 to 5 × 10^{7} C.P.M. of C^{14}. The rats were sacrificed 15 minutes after the C^{14} injections, and their entire livers were excised and analyzed for C^{14}-cholesterol.

The values for the rats fed the non fat diet ranged from 0.35 to 0.42 per cent of the injected C^{14} per whole liver, and those for the fat-fed rats ranged from 0.71 to 0.77 per cent.

DISCUSSION

We showed earlier that the amount of carbohydrate ingested by the rat—or apparently the extent of glycolytic activity in the liver—influences the liver's capacity for incorporating acetate carbon into cholesterol (1, 8). For this reason, in the present study, care was taken to insure that, whether the diet contained no fat or 15 per cent fat, the proportion of glucose remained constant, namely, 50 per cent. It is therefore not surprising that the concentrations of glycogen in the livers of the rats fed the 0 and 15 per cent fat diets were essentially the same. Since the variations in protein content of the diet (necessary to keep the caloric value per unit weight of the diets constant) had no measurable effect on hepatic cholesterogenesis, our results demonstrate that the liver's capacity for incorporating acetate carbon into cholesterol was related to the quantity of fat in the diet.

Under conditions of fat feeding identical with those used here, we have observed a lowering in the liver's capacity for synthesis of fatty acids (24). Indeed, lipogenesis from acetate is extremely sensitive to fat feeding—the addition of as little as 2.5 per cent fat to the diet decreased the liver's capacity for incorporating acetate carbon into fatty acids. Thus, when fat is fed, hepatic lipogenesis and cholesterogenesis are inversely affected. A similar inverse relation between cholesterogenesis and lipogenesis has been observed in the liver of the diabetic rat (25, 7). These observations indicate that hepatic cholesterogenesis, like acetoacetate formation (26), may represent an alternate pathway for acetyl CoA when lipogenesis is impaired under certain conditions.

Previous work dealing with the effect of dietary fat on cholesterol synthesis has yielded results differing from those observed here. Alfin-Slater *et al.* (27) studied the synthesis of cholesterol in intact rats which received deuterium oxide after they had been fed diets containing either 2.2 or 30 per cent fat. According to these investigators, the amount of newly formed cholesterol present in the liver and plasma of rats prefed a low fat diet is not changed by feeding them a high fat diet. The results obtained by Brice and Okey (28) with C^{14}-acetate were too variable to enable them to state whether rats fed 5 per cent fat differed, in their ability to synthesize cholesterol, from rats that had been fed a diet containing 40 per cent fat for 3 weeks.

Recent work on man and animals suggests that saturated and unsaturated fatty acids differ in their effects upon cholesterol metabolism (12, 13, 15). In this connection it is of interest to note that one aspect of cholesterol metabolism in the rat, namely, its synthesis in liver, responded in a uniform manner

to the feeding of four fats: lard, corn oil, a vegetable oil, and a hydrogenated vegetable oil.

SUMMARY

Rats were fed, for 3 days, four synthetic diets, all of which contained the same proportion of carbohydrate (50 per cent) and were of equal caloric value per gram. These diets contained either 0 or 15 per cent fat. The fats used were lard, corn oil, Wesson oil, and a hydrogenated vegetable oil. The feeding of the fat-containing diet for 3 days increased the liver's capacity for incorporating acetate carbon to cholesterol. The fats tested were of about equal value in stimulating hepatic cholesterogenesis.

The various diets fed had no effect on the lipide or glycogen content of the liver nor on the lipide content of plasma.

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CHARACTERISTICS OF CANADIAN LARD[1]

By H. J. Lips[2*] and G. A. Grant[3]

Abstract

Mean values for measured properties of 33 samples of lard obtained from 26 packing plants across Canada were: iodine number, 58.7; saponification number, 193.9; melting point, 43.5° C.; smoke point, 382° F.; colour, 8.8Y, 1.6R; unsaponifiable matter, 0.43%; fatty acid composition: saturated, 45.6%, oleic, 44.7%, linoleic, 8.7%, linolenic, 0.6%, arachidonic, 0.4%; storage life at 26.7° C., 9.2 weeks; Swift stability, 3.5 hr.; iodimetric peroxide, 1.6 ml. of 0.002 *N* thiosulphate per gm.; ferrometric peroxide, 9.7 m.e. per kgm.; Kreis test, 9.9; Stamm test, 2.3; alpha-dicarbonyl test, 3.4; free fatty acid, 0.4%; fluorescence, 79.2. The distribution of values is shown by histograms.

Simple correlation coefficients computed between measured properties showed the following to be associated: melting point with iodine number; free fatty acid content with melting point, smoke point, and red colour; storage life at 26.7° C. with log of Swift stability and initial ferrometric peroxide, Kreis, and alpha-dicarbonyl values.

Introduction

In pre-war years there was usually a lard surplus because the housewife preferred hydrogenated vegetable shortening to the animal product. The factors that have contributed to the inferior competitive position of lard are: lack of resistance to spoilage, lack of blandness, and unsuitability for use in deep-fat frying due to a low smoke point (28, 42). However, in pastry the shortening power of lard is often superior to that of hydrogenated vegetable oils (41, 58).

In the United States, progress has been made in the development of bland lards that may be kept for extended periods without refrigeration. These products owe their desirable characteristics to special processing, including: deodorization, partial hydrogenation, addition of hardened lard "flakes", or the incorporation of antioxidants such as gum guaiac and lecithin (44, 49, 61). In Canada, although the Food and Drugs Act has been amended to permit the incorporation in lard of certain antioxidants, commercial stabilization under the Act has not begun. Provided that additions are indicated on the container and that not more than 0.2% of stabilizing material is added, singly or in combination, the following substances may now be used: gum guaiac; vegetable oil containing tocopherols; lecithin; citric, tartaric and ascorbic acids (12).

Formerly Canada imported over 50% of her fats and oils requirements, exclusive of butter (35). During the war, the lard industry expanded, owing to a large increase in hog production and to emergency salvage and carcass

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trimming operations. It is expected that there will be an eventual surplus of lard, and one of the problems confronting the Canadian packing industry will be the improvement of this product so that high levels of export and domestic consumption can be maintained in the face of post-war competition by shortenings prepared from vegetable oils.

As a basis for proposed work to improve the quality of Canadian lard, it was considered advisable to determine the characteristics of the product as freshly produced. Surveys of Canadian lard were conducted as early as 1888 by the Food and Drugs laboratory (then a branch of the Department of Inland Revenue), but these were concerned only with adulteration of lard in respect to other fats and excess moisture (30).

A preliminary examination of market samples obtained in the city of Ottawa in the summer of 1944 showed an average keeping time of only five weeks at 26.7° C. With this indication of stability as a guide a more extensive survey of available commercial products was begun in September, 1944. Questionnaires concerning plant practice were submitted to Canadian packing houses, and representative samples of processed material were requested.

Materials and Methods

A total of 33 samples of lard was obtained from 26 packing plants across Canada and stored at −40° C. until required. Of these samples 12 were dry rendered and 21 wet rendered. As indicated in replies to questionnaires, the details of processing procedure varied considerably from plant to plant.

The product was characterized by determination of Kaufmann iodine number (14, 26); saponification number (24); capillary melting point (5); unsaponifiable matter (2); oleic, linoleic, linolenic, and arachidonic acids (9); smoke point (2); Lovibond colour (2); free fatty acid (32); and Swift stability* (27, 40). Organoleptic storage life at 26.7° C.; iodimetric and ferrometric peroxide oxygen; Kreis, Stamm, and alpha-dicarbonyl tests; and fluorescence were determined by methods reviewed elsewhere (18).

Results and Discussion

It was necessary to respect the confidential nature of some of the data obtained from the questionnaires but the majority of the laboratory results are reported (Fig. 1 and Table I). Initial odour is not given, but should be mentioned because many samples were objectionable in varying degree, having definite 'porky', 'tankage', or 'burnt' odours. Lovibond blue reading and initial iodimetric peroxide content are not represented graphically, as the results included a number of zero values. No histogram for arachidonic acid concentration is given because the variation in value was slight.

* *When the indicator method (40) is used to determine Swift stability, the indicator should be renewed after the volatile fatty acids originally present have been taken off. This should not take longer than* [illegible]

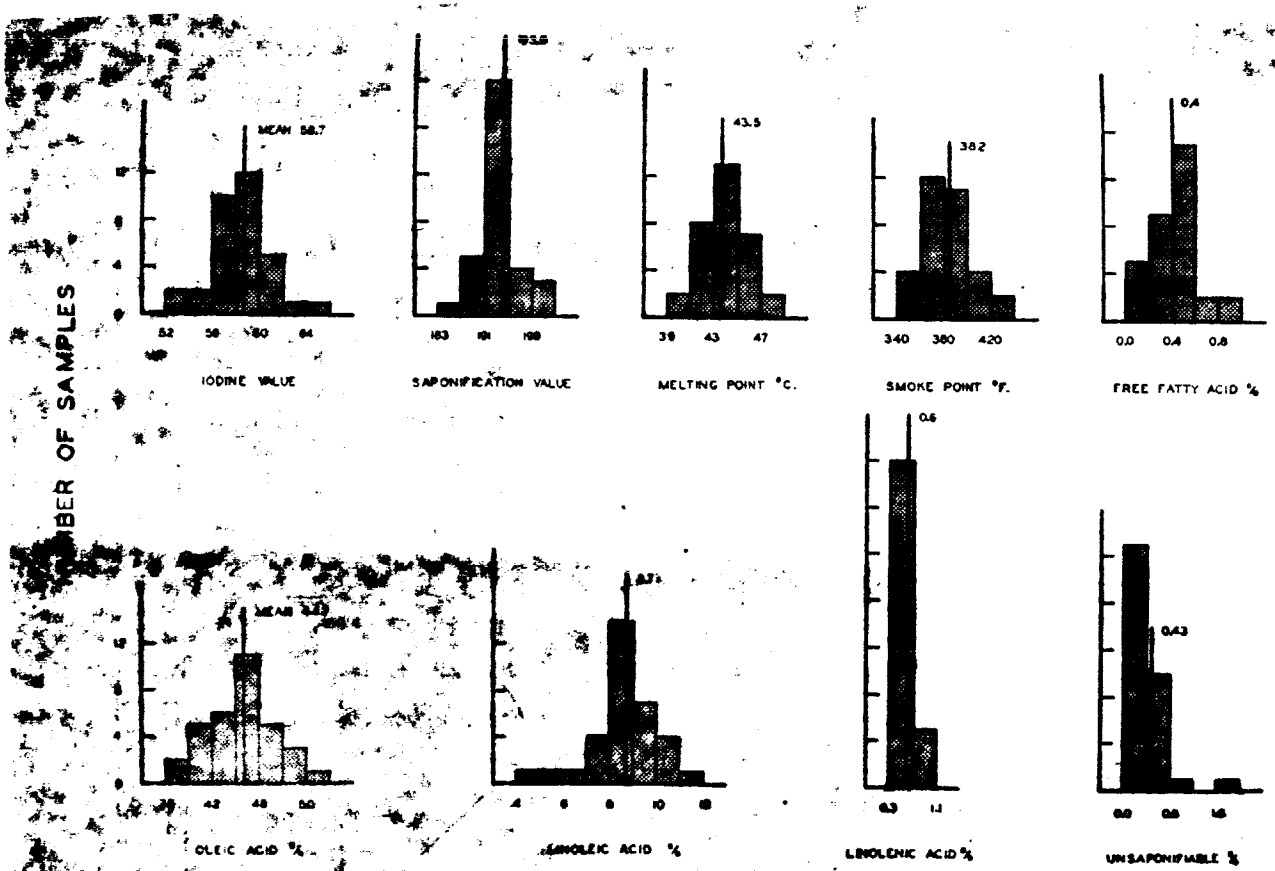

Fig. 1. Distribution of measured properties of Canadian lard.

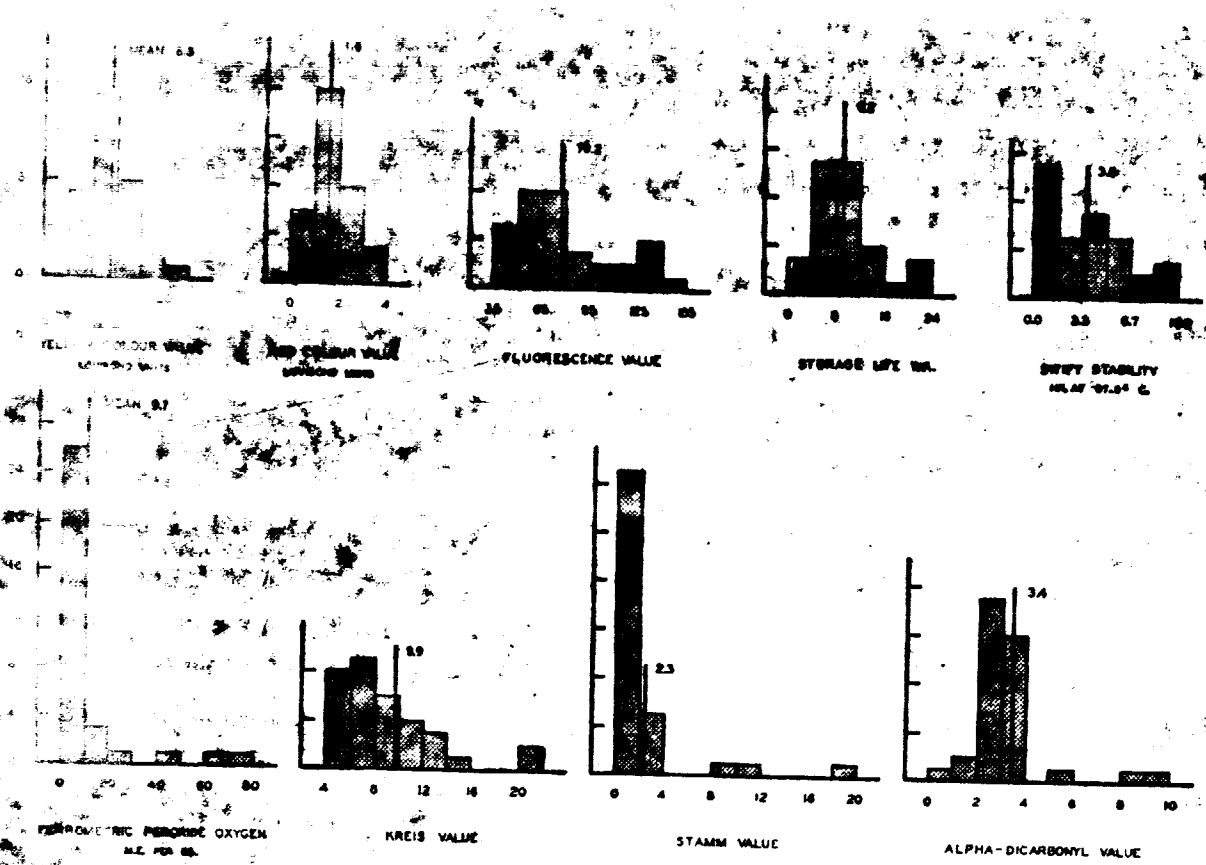

FIG. 1b. *Distribution of measured properties of Canadian lard.*

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TABLE I

MEAN VALUES AND RANGES FOR MEASURED PROPERTIES OF CANADIAN LARD

Measurement	Mean	Range	Measurement	Mean	Range
Iodine number	58.7	53.1–65.3	Swift stability, hr. at 97.8 °C.	3.5	0.3–9.0
Saponification number	193.9	184.2–202.2	Ferrometric peroxide, m.e. per kgm.	9.7	0.0–73.2
Melting point, °C.	43.5	39.3–47.9	Iodimetric peroxide, ml. of 0.002 *N* thiosulphate per gm.	1.6	0.0–14.2
Smoke point, °F.	382	345–438	Kreis test, extinction coefficients	9.9	3.7–20.7
Yellow colour, Lovibond units	8.8	3.0–22.3	Stamm test, extinction coefficients	2.3	0.0–19.9
Red colour, Lovibond units	1.6	0.4–3.1	Alpha-dicarbonyl test, extinction coefficients	3.4	0.8–9.9
Blue colour, Lovibond units	0.2	0.0–1.2	Free fatty acid, as % oleic	0.4	0.1–0.9
Saturated acids*, %	45.6	—	Fluorescence, photofluorometer units	79.2	39.8–146.0
Oleic acid*, %	44.7	38.6–51.8			
Linoleic acid*, %	8.7	4.6–11.3			
Linolenic acid*, %	0.6	0.4–1.1			
Arachidonic acid*, %	0.4	0.3–0.5			
Unsaponifiable matter, %	0.43	0.20–1.70			
Storage life, weeks at 26.7° C.	9.2	2.0–22.0			

**Expressed as % of total acids.*

Characteristics of Canadian Lard

Values for iodine number, saponification number, and melting point had a fairly normal distribution (Fig. 1). Variation in these values, in the absence of adulteration or advanced spoilage, would seem to be chiefly due to differences in the original fat before processing.

There was appreciable variation in oleic, linoleic, and linolenic acid concentrations as determined spectrophotometrically. Saturated acid values were obtained by difference only. Here again the differences were concerned with the original raw materials.

Values for unsaponifiable matter vary to some extent with the methods of rendering the lard, and large variations may affect the values obtained for other measurements on the whole fat (26, pp. 401, 425). The values greater than 0.8% are unusually high.

It is considered that the smoke point of lard should be at least 400° F. to compete with that of shortening, since a survey of 17 wartime shortenings, including animal-vegetable compounds, showed a mean value of 424° F. (30).

For several samples it was necessary to remove suspended material by filtration before colour measurements could be made. Blue readings were obtained for 10 of the 33 samples, and it was found that the presence of carbon in suspension markedly increased the apparent blue colour. Variations in colour would appear to be chiefly governed by factors of processing technique.

Stability of most of the lard samples was poor, as estimated by storage at 26.7° C. and by Swift stability at 97.8° C. The magnitude of these measurements depends on the nature of the original material and the [illegible] procedure, if it is assumed that no antioxidants were [illegible]

The values for iodimetric peroxide, ferrometric peroxide, Kreis test, Stamm test, alpha-dicarbonyl test, and free fatty acid reflect the degree of exposure of the lard during processing to spoilage by biological and chemical action. Considerable variation in these respects is shown in Table I and Fig. 1. Fluorescence values were unevenly distributed.

Interrelation of Measured Properties

The literature contains a number of references to relations between various measured properties of fats and oils. Iodine value is reduced by marked oxidation (33, p. 95; 36, p. 425), saponification number is influenced to some extent by free fatty acid content (36, p. 394), and melting point is appreciably altered by changes in free fatty acid (36, p. 329). Moisture content, by affecting lard transparency, may influence estimation of the capillary melting point (17; 36, p. 288). However, according to the Canadian Food and Drugs Act (12), moisture content of lard is limited to 1%, and the product must be free from rancidity. In the absence of changes in composition, smoke point varies inversely with free fatty acid content (7; 23, pp. 132-136).

In the present study the relation of measured properties to one another was investigated by calculating simple coefficients of correlation, except where this was precluded by the presence of a large number of zero values, as in the case of iodimetric peroxide and Lovibond blue measurements. Most of the correlations proved to be insignificant; only the more important ones are reported (Table II).

Iodine number was inversely related to melting point, but no association of saponification number with other measured properties was found. The significant association of melting point with free fatty acid content indicates that the free fatty acids formed before or during processing lowered the melting point.

Smoke point, Lovibond red colour, and free fatty acid content were all interrelated. High values for free fatty acid were associated with low smoke point and high red colour. Lowering of the smoke point by free fatty acids is a well recognized effect, and significant correlations have been reported (42). The relation of free fatty acid to red colour suggests that there was a parallel increase in these two measurements during processing. Red and yellow colour readings were directly related.

The correlation of storage life at 26.7° C. with log Swift-stability (Fig. 2) indicates that the latter determination has certain usefulness in the prediction of lard keeping quality at lower temperatures, at least in the absence of added antioxidants. There has been some question about this point (51). Storage life varied inversely with ferrometric peroxide, Kreis and alpha-dicarbonyl values; that is, samples with high values for these chemical measurements had short storage lives, a[illegible] oxidative deterioration was already in progress. Iodine value, content of [illegible]turated and unsaturated acids and unsaponifiable matter, and fluorescence [illegible] no observed important relation to storage life. The results support a [illegible] suggestion that degree of

unsaturation in pigs' fat is not necessarily the limiting factor in determining stability (34, pp. 60-64).

Other relations examined between measured properties were not significant.

TABLE II

COEFFICIENTS OF CORRELATION BETWEEN MEASURED PROPERTIES OF CANADIAN LARD

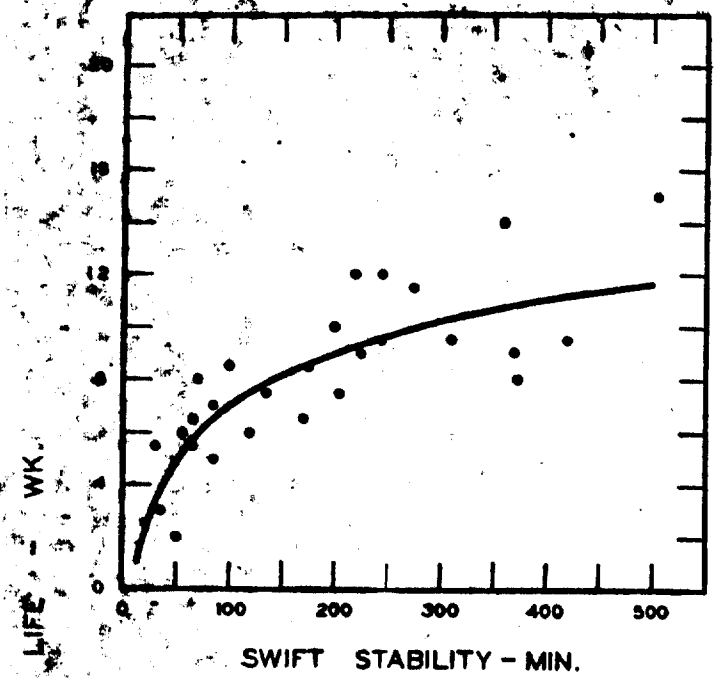
Quantities correlated	No. of values	Correlation coefficient
Iodine number with:		
Melting point	33	− .41*
Melting point with:		
Smoke point	33	.41*
Lovibond yellow	33	− .29
Lovibond red	33	− .24
Free fatty acid	33	− .59**
Smoke point with:		
Lovibond yellow	33	− .19
Lovibond red	33	− .38*
Free fatty acid	33	− .87**
Lovibond yellow with:		
Lovibond red	33	.82**
Lovibond red with:		
Free fatty acid	33	.41*
Storage life with:		
Log Swift stability	29	.83**
Ferrometric peroxide	26	− .63**
Kreis test	29	− .39*
Stamm test	27	− .28
Alpha-dicarbonyl test	27	− .36*

* *Indicates 5% level of statistical significance.*
** *Indicates 1% level of statistical significance.*

Relation of Measured Properties to Plant Practice

Stability and smoke point were considered likely to be altered by variations in processing procedure, so the effect of these variations upon storage life and smoke point was assessed on wet and dry rendered lards considered together and separately (Table III). However, the calculated correlation of any one plant practice with the measured properties is not strictly valid, as all other factors were not constant among plants. Moreover the calculations are based on a limited number of values, and the results may not reflect general operational experience in all cases.

A preliminary analysis of variance demonstrated no important difference in mean storage life between wet and dry rendered lard, but showed that the mean smoke point for dry rendered lard was significantly higher than for the wet rendered product. The latter result is attributable to the higher free fatty acid content of the wet rendered fats, due to their exposure to the hydrolyzing effect of long contact with steam or hot water.


LIFE - WK.
0
4
8
12
16
20
0
100
200
300
400
500
SWIFT STABILITY - MIN.

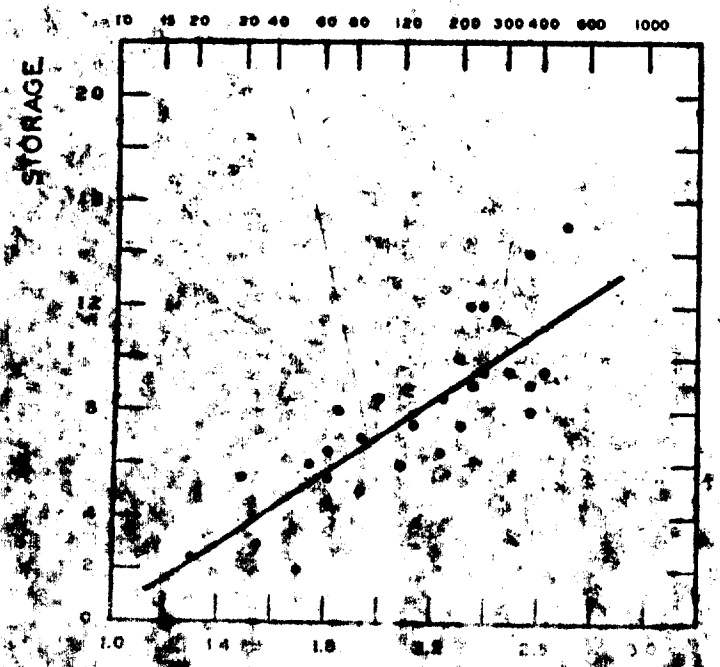

10 15 20 30 40 60 80 120 200 300 400 600 1000
STORAGE
0
2
4
6
8
12
16
20
1.0
1.4
1.8
2.2
2.6
3.0
LOG. OF SWIFT STABILITY

TABLE III

COEFFICIENTS OF CORRELATION BETWEEN PLANT PRACTICES AND MEASURED PROPERTIES FOR CANADIAN LARD

Quantities correlated	No. of values			Correlation coefficients		
	Wet rendered lard	Dry rendered lard	Total	Wet rendered lard	Dry rendered lard	Total
Storage life with:						
Killing fat, %	21	12	33	.15	.10	.14
Cutting fat, %	21	12	33	− .14	− .35	− .23
Bones, %	21	12	33	.77**	− .15	− .22
S.P.† material, %	21	12	33	− .02	− .31	− .11
Rendering temperature	21	12	33	.41	− .30	− .12
Rendering time	3	11	14	− .42	.69*	− .02
Deodorizing temperature	—	4	5	—	.00	− .40
Deodorizing time	—	4	5	—	− .70	.08
Filtering temperature	20	11	31	− .31	− .43	− .32
Time, processing to packaging	21	12	33	.10	− .30	− .12
Smoke point with:						
Killing fat, %	21	12	33	− .77**	.52	− .19
Cutting fat, %	21	12	33	− .15	.22	− .09
Bones, %	21	12	33	.40	.53	.33*
S.P.† material, %	21	12	33	− .03	.20	.11
Rendering temperature	21	12	33	.33	.82**	.51**
Rendering time	3	11	14	.99*	− .56	− .71**
Deodorizing temperature	—	4	5	—	− .88	− .45
Deodorizing time	—	4	5	—	− .83	− .42
Filtering temperature	20	11	31	.32	− .12	− .22
Time, processing to packaging	21	12	33	− .10	.19	− .08

† *Sweet pickle*
* *Indicates 5% level of statistical significance.*
** *Indicates 1% level of statistical significance.*

The storage life of wet rendered lard appeared to be lengthened by increase in amount of bones (Table III), presumably because of their content of natural stabilizing substances. The presence of 0.14% lecithin in pig-bone fat as compared to 0.04 to 0.06% in lard has been reported (25). With the dry rendered lard, increase in rendering time appeared to be beneficial. This may be related to better extraction of natural stabilizers from the non-fatty material. Although correlations of storage life with filtering temperature failed to attain statistical significance, they were all negative, indicating that moderately low filtering temperatures (160° to 170° F.) would be desirable for the production of more stable lard.

High smoke point was favoured by decrease in killing fat in wet rendering and by an increase in bones. A negative correlation of smoke point with cutting fat rather than killing fat had been anticipated, since the former fat usually has more time to develop free fatty acids by enzymic action before rendered (23, pp. 132–136; 37, 45, 60). Similarly it was expected that

increase in bones might lower the smoke point, since these may stand for some time before rendering, with consequent development of free fatty acids in the fat that they carry. No satisfactory explanation can be offered for these discrepancies at present because of limited information about the operations concerned. The relation between smoke point and rendering temperature was positive, and between smoke point and rendering time negative. This suggests that rendering should be carried out at a relatively high temperature in as short a time as possible (23, pp. 132–136; 43, 55).

Although none of the other relations tabulated was significant, reduction in deodorizing temperatures and times used appeared to be desirable. In general the influence of processing procedures upon wet and dry rendered products was dissimilar.

Comparison with Lards from Other Countries

Extensive control examinations of lard in other countries have been reported (1, 13).

Comparative figures in the literature on the composition of lard are largely based on chemical rather than spectrophotometric measurements. For 27 American lards (4), the calculated compositions were predicated on the absence of fatty acids more unsaturated than linoleic (Table IV). However, the presence of linolenic, unsaturated C_{20-22}, and conjugated acids has been demonstrated (10; 31; 21, pp. 80–86). The presence of arachidonic acid is used as the basis of a spectrophotometric method for distinguishing between lard and hydrogenated vegetable oils (6). The greater unsaturation of other lards as compared to Canadian lards is undoubtedly due in part to differences in the nature and fat content of the diet of the hog (33, pp. 17–26). Changes in the diet similarly affect melting point, but have less effect on saponification number.

Comparative figures for lard colour have not been found in the literature. Authentic blueness in lard is said to be due to a natural pigment, and is considered an indication of careful processing rather than poor handling.* The blue pigment is unstable, and on oxidation it changes to red, or a mixture of red and yellow (23, pp. 157–158). Its presence is objectionable, since the consumer prefers whiteness.

The smoke point figure quoted in Table IV for American samples does not include the value given for a bland lard (59).

Available figures for small numbers of American samples indicate that lard usually has indifferent keeping quality unless it is stabilized by the addition of antioxidants, or subjected to special processing treatments (50).

The use of fluorescence in the study of fats to detect impurities and deterioration has been suggested by several workers (19, 20, 47, 54) but no recent data are available for comparison. Fluorescence in lard is said to depend both on state of preservation and manner of processing (8, 16, 48, 53).

TABLE IV

A COMPARISON OF VALUES FOR MEASURED PROPERTIES OF CANADIAN LARD WITH OTHER REPORTED AND RECOMMENDED VALUES

Measurement	Comparative values			
	Canadian		American	Other
	Mean	Range		
Iodine number	58.7	53.1–65.3	27 samples, 64.6 (4) Recommended, 46–70 (3)	General, 46–66 (22, p. 136) English, 108 samples, 57–73 (56) Portuguese, 30 samples, 63.9 (46)
Saponification number	193.9	184.2–202.2	Recommended, 195–202 (3)	General, 193–200 (22, p. 136) Portuguese, 30 samples, 195.8 (46)
Melting point, °C.	43.5	39.3–47.9	—	General, 28–48 (22, p. 136)
Smoke point, °F.	382	345–438	6 samples, 371 (59)	—
Saturated acids*, %	45.6	—	27 samples, 36.8 (4)	Portuguese, 30 samples, 38.5 (46)
Oleic acid*, %	44.7	38.6–51.8	27 samples, 51.5 (4)	Portuguese, 30 samples, 49.2 (46)
Linoleic acid*, %	8.7	4.6–11.3	27 samples, 11.7 (4)	Portuguese, 30 samples, 12.5 (46)
Arachidonic acid*, %	0.4	0.3–0.5	0.02–0.21 (15) 0.31–0.40 (11) 0.2 –0.6 (6)	
Unsaponifiable matter, %	0.43	0.20–1.70	Recommended, 1.0 max. (3)	General, 0.2–0.4 (22, p. 136; 29, p. 135) Portuguese, 30 samples, 0.14 (46)
Swift stability, hr. at 97.8 °C.	3.5	0.3–9.0	7 samples, 5.3 (59)	Other Canadian, 14 samples, 2.2 (38, 40)
Iodimetric peroxide, ml. of 0.002 *N* thiosulphate per gm.	1.6	0.0–14.2	4 samples, 1.5 (28) 6 samples, 0.7 (52) Recommended, 2.5 ml. max. (3)	—
Free fatty acid, as % oleic	0.4	0.1–0.9	6 samples, 0.26 (59) 5 samples, 0.29 (28) 3 samples, 0.61 (57) 4 samples, 0.35 (28) Recommended, 1% max. (3)	General, 0.2–0.7 (22, p. 136)

* *Expressed as % of total acids.*

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Fatty Acids of Lard. B. Quantitative Estimation by Silicic Acid and Gas-Liquid Chromatography[1]

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Abstract

A quantitative comparison was made of the fatty acid composition of a commercially rendered lard, a laboratory rendered lard, and a solvent extracted lard representing the same batch of pig tissue. The composition was determined by a preliminary separation of the methyl esters on a silicic acid column followed by gas-liquid chromatography of the fractions containing a known added amount of reference compound. This technique permitted estimation of the composition of each fraction and subsequent calculation of percentage of the minor as well as the major components in the original lard. It was concluded that the minor components were not artifacts produced in rendering.

Introduction

IMPROVED TECHNIQUES in application of gas-liquid chromatography (GLC) for separating fatty acid esters have shown that natural fats are more complex than was previously reported. Numerous minor components are being found which had previously escaped detection. However, some natural fats (1,2,4, 6,7,9,10) contain such a multiplicity of minor components, many of which have essentially the same retention on a given polyester column, that even the use of several columns with different stationary phases will fail to resolve and permit detection of all the components. Hence, some auxiliary means of fractionation followed by gas-liquid chromatography on each of these fractions is sometimes necessary. Furthermore, some fatty acids are present in such low concentrations that preliminary fractionation is required to effect sufficient concentration for detection by GLC.

In recent work from this laboratory on fatty acids of lard (Part A) (3) attention was given primarily to identification of minor fatty acids in lard. A nonpolar silicone column was employed to separate the fatty acid methyl esters mainly on the basis of chain length. Fractions were collected and rechromatographed on a polyester (EGS) column. This technique permitted detection of many minor or trace components which had not been reported in previous work. No attempt, however, was made to estimate percentage distribution owing to uncertainties concerning quantitation in collection of fractions.

In the present work attention was given to quantitative aspects. Preliminary fractionation of the lard methyl esters was made by silicic acid column adsorption chromatography. The fractions thus obtained, after the addition of a known amount of reference ester to each, were then subjected to GLC on a polyester column. The percentage fatty acid distribution in the total lard, determined by summation of data from the fractions, is presented for three lards obtained from the same batch of tissues. The same fatty acids were found in each specimen in about the same order of percentage. It was concluded that the minor components were not artifacts produced in rendering.

Experimental

Samples. A fresh sample of steam-rendered lard was obtained from a local packer on the same day it was rendered. Fresh pig tissues representative of the commercial batch being rendered were also obtained and were frozen until they could be processed in the laboratory. The tissues were minced, mixed, and divided into two parts. One part was carefully rendered in a glass vessel under nitrogen at 115-120C; the other was thoroughly extracted with redistilled acetone, methylal-methanol (4:1), and hexane at room temperature. The solvents were removed from the filtered extracts in a rotary evaporator under reduced pressure.

The commercially rendered lard and the two corresponding lard samples produced in the laboratory were converted to methyl esters, and freed of unsaponifiables by silicic acid column treatment (8).

GLC Apparatus. The apparatus and thermal conductivity cell detector employed were the same as described previously (5).

GLC Columns. The separations were performed at a temperature of 212C with a stainless steel coiled column 8 ft × $^3/_{16}$ in. OD (0.118 in. ID) and packed with acid and base washed Chromosorb "W" (42-60 mesh) coated with 25% ethylene glycol succinate polyester. The treatment of the support and preparation of the polyester has been described (4).

Adsorption Columns. A slurry of 3.5 g of 80% silicic acid-20% filter aid in hexane was poured into a conventional chromatographic column 12 mm × 250 mm. When the packing was properly settled, 30-40 mg of lard methyl esters were introduced onto the column and eluted. Three fractions were obtained by elution with 50 ml each of hexane and a final fraction eluted with 100 ml of an equal mixture of hexane and ethyl ether.

Quantitation. A known amount of highly purified 15:0[3] methyl ester was added to each fraction from the adsorption column separation and then each was subjected to GLC analysis. The area under the peak on the chromatogram of the individual components in each fraction was measured and made relative to that of the reference ester. Since the area representing the reference ester is equivalent to a known weight, the weight of each component could be calculated. When these weights from all of the fractions were summated the result was the total weight of lard methyl esters and from these data percentages were calculated. The composition of the lard methyl esters was also determined directly from the areas of the peaks on the chromatogram of the total esters without the use of an internal standard.

[1] Presented in part at the AOCS meeting, St. Louis, Mo., 1961.
[2] Eastern Utiliz. Res. & Dev. Div., ARS, U.S.D.A.

[3] A "shorthand" designation of structure of each fatty acid is used throughout this paper. 18:0 = saturated C_{18} acid; 18:2 = C_{18} with double bonds; 18:0 br = branched chain C_{18} acid, etc.

Results and Discussion

In an earlier publication (5), it was not possible to determine tetraenes and pentaenes by GLC on the total esters of lard with the column and conditions employed; however, with the present narrower diameter column and operating conditions it was possible to estimate the amount of 20:4 (arachidonic acid) but not the 20:5 nor the unsaturated C_{22} acids. Chromatograms of the total methyl esters of the three lards were essentially identical and are represented by Figure 1. The chromatogram was obtained by injecting a relatively large sample, actually a slight overload, in order to show the presence of many of the minor components. Notice that the peak due to the presence of a mixture of 14:1 and 15:0 (peak 4) can readily be seen although together they amount to only about 0.03%. By way of contrast, about seven times as much of 20:4 (peak 15) is difficult to detect since its peak, occurring later on the chromatogram, is broad but has very little height owing to its dilution with carrier gas as it slowly moves off the column. For the same reason, none of the C_{22} esters are seen. The C_{19} esters are completely coincident with the peaks of the C_{18} unsaturated esters.

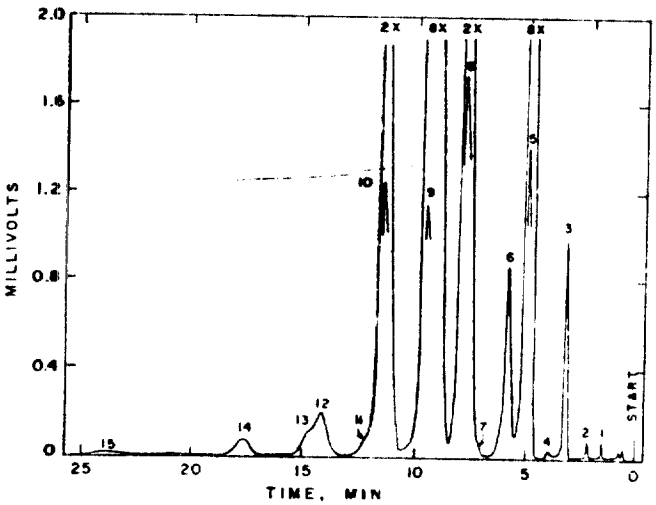

FIG. 1. Chromatogram of the total esters of lard. Column 8 ft × 3/16 in., temp 212C, 25% EGS polyester. Peak identification: 1. 10:0; 2. 12:0; 3. 14:0; 4. 14:1 + 15:0; 5. 16:0; 6. 16:1 + 17:0; 7. 17:1; 8. 18:0; 9. 18:1; 10. 18:2; 11. 20:0; 12, 20:1; 13. 18:3; 14. 20:2; 15. 20:4. 2X and 8X = attenuation.

In order to separate esters with similar retentions by GLC and to concentrate the minor components, it was necessary to employ auxiliary fractionation. In previous work on milk fatty acids (4), silicic acid adsorption chromatography was shown to be effective for this purpose. It was employed successfully in the present investigation. Four fractions were obtained, the first, amounting to approximately 1/3 of the 30–40 mg sample applied to the column, consisted mainly of saturated esters. Qualitative evidence was also found for traces of hydrocarbons in this fraction. The second fraction, about 1/2 the sample, consisted principally of saturated and monounsaturated esters. The balance of the sample was about equally distributed in a third and fourth fraction; the third contained the monounsaturated plus diunsaturated esters and the fourth the diunsaturated plus the more highly unsaturated esters.

It was not necessary to weigh each fraction from the silicic acid separation since the addition of a known quantity of a reference ester as an internal standard to each fraction permitted calculation of the amount of each component from the gas-liquid chromatogram. The center of a chromatogram is the optimum position for a peak representing the internal standard. Owing to the multiplicity of components in lard, this position was not suitable and the 15:0 methyl ester, even though appearing early on the chromatogram, was chosen because it offered the least interference in the calculation of the fatty acid components. Although 15:0 is present in lard, as shown on the chromatogram of the total esters (Fig. 1, peak 4) it occurs only to the extent of 0.02 or 0.03% and therefore does not materially affect the results. We added the internal standard to each fraction in an amount equal to 5–10%.

The peaks on the chromatograms were identified by comparison with reference esters and from a plot of carbon chain lengths for a homologous series of compounds against the logarithm of their relative retention times. Each fraction was chromatographed before and after hydrogenation as a further check on components believed to be unsaturated esters. All acids reported in lard in the previous publication (3) were found in this study in an amount such that a quantitative figure could be assigned to them with the exception of 22:0, 22:1, 22:2, and 22:3. These acids are present in such small amounts that additional concentration of them would be necessary for a quantitative assignment.

The composition of the three lards determined from GLC analyses of the fractions obtained from the silicic acid column separation are shown in Table I along with GLC analyses based on the chromatograms of the total lard esters. For those components that appear on the chromatograms of the total lard esters, the percentage values show reasonable agreement with those obtained by summation of analyses of their fractions. Obviously more components were determined

TABLE I

Composition of Three Lards from GLC Analysis on Total Esters and from Fractions Obtained by Silicic Acid Separation

Component	Commercial lard		Lab. rendered lard		Solv. extracted lard	
	Total[a]	Fraction[b]	Total[a]	Fraction[b]	Total[a]	Fraction[b]
	%	%	%	%	%	%
10:0	0.04	0.01	0.05	0.02	0.04	0.01
12:0	0.07	0.04	0.06	0.05	0.06	0.05
14:0	1.17	1.09	1.17	1.11	1.24	1.06
14:1	0.06	0.02	0.03	.....	0.04	0.01
15:0		0.02		0.02		0.03
16:0	26.4	25.7	26.2	24.8	27.0	27.6
16:1	2.64	2.20	2.16	1.87	2.04	1.71
17:0		0.45		0.21		0.46
17:1	0.28	0.22	0.20	0.20	0.21	0.10
18:0 br	.....	trace	.....	trace	.....	trace
18:0	10.2	12.0	12.5	13.3	11.6	14.4
18:1	47.3	45.2	46.4	45.1	46.5	42.0
18:2	9.52	10.4	9.05	10.6	8.98	9.93
conj. 18:2 *c,t*[c]	.....	0.18	.....	0.09	.....	0.09
conj. 18:2 *t,t*[c]	.....	0.08	.....	0.04	.....	0.04
18:3	0.52	0.34	0.49	0.41	0.56	0.42
19:0	.....	0.04	.....	0.03	.....	0.04
19:1	.....	0.12	.....	0.07	.....	0.08
20:0	0.37	0.18	0.32	0.17	0.35	0.20
20:1	0.93	0.72	0.87	0.87	0.93	0.85
20:2	0.37	0.37	0.40	0.45	0.39	0.35
20:3	trace	0.11	trace	0.07	trace	0.09
20:4	0.16	0.20	0.20	0.20	0.15	0.21
20:5	.....	0.09	.....	0.05	.....	0.09
22:0	.....	trace	.....	.....	.....	0.01
22:2	.....	0.02	.....	.....	.....	.....
22:4	.....	0.05	.....	0.05	.....	0.05
22:5	.....	0.04	.....	0.03	.....	0.02
Unknown	.....	0.03	.....	0.02	.....	0.07

[a] Calculated from a chromatogram of the total lard methyl esters
[b] Calculated from chromatograms of fractions obtained by silicic acid column separation of lard methyl esters and use of internal standard.
[c] *c,t* and *t,t* = *cis,trans* and *trans,trans*.

on these ester fractions than was possible on the total lard esters. The greatest numerical variations in percentages are those for methyl stearate and methyl oleate. This may be due mainly to normal experimental errors in measuring the peak areas, since one or the other of these esters is present in three fractions in amounts requiring attenuation of the detector signal. It should be noted that although one lard was commercially rendered, another rendered in the laboratory, and the third solvent extracted each contained the same components. It is concluded, therefore, that the components are naturally occurring and not produced as artifacts in the rendering process.

A comparison of the polyunsaturated acids found in the three lards as determined by GLC on the fractions and by the ultraviolet spectrophotometric method on the total esters is shown in Table II. Good agreement was obtained even for the tetraenes and pentaenes. The small differences in composition between the three lards by the fractionation procedure are not considered significant and probably are the result of normal errors involved in measuring peak areas and in adding the internal standard. The use of auxiliary fractionation by silicic acid and the use of GLC on the fractions obtained affords a means of quantitative estimation of minor components as well as major components of complex fats.

TABLE II

Polyunsaturated Acid Composition of Three Lards by GLC and U.V. Spectrophotometry

	Commercial lard		Lab rendered lard		Solv. extracted lard	
	U.V.[a]	GLC[b]	U.V.[a]	GLC[b]	U.V.[a]	GLC[b]
	%	%	%	%	%	%
Diene	12.2	11.1	11.6	11.2	11.6	10.4
Triene	0.87	0.15	0.78	0.51	0.89	0.51
Tetraene	0.35	0.25	0.26	0.24	0.27	0.26
Pentaene	0.15	0.15	0.11	0.09	0.12	0.12

[a] Determined on total lard methyl esters.
[b] Calculated from chromatograms of fractions obtained by silicic acid column separation of lard methyl esters and use of internal standard.

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Effects of Feeding Heated Lard to Rats, With a Histologic Description of the Lesions Observed

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INTRODUCTION

Available statistics indicate that cancer of the digestive tract in man accounts for approximately 45 percent of all deaths from cancer at the present time. It seems logical to suspect dietary constituents of being involved in the genesis of this disease, since food and its digestive products come into intimate contact with the tissues lining the alimentary canal.

Many investigators have attempted to produce gastric neoplasms in experimental animals by dietary means. In 1913 Singer (*1*) described the production of gastric lesions in a high percentage of his experimental rats and tentatively ascribed an infectious origin to these lesions. Apparently unsuspected by Singer, the nutritionally poor diet which he fed his animals may have been an important factor. Similarly, the lesions in the stomachs of nematode-infested rats described by Fibiger (*2*) are of uncertain etiology. In repeating Fibiger's experiments, Passey, Leese, and Knox (*3*) found that rats maintained on Fibiger's diet of white bread and water developed some gastric lesions even in the absence of nematode infestation, although these lesions were less severe than those seen in a similar group infested with *Gonglyonema neoplasticum*. Other rats carrying the nematode, on a complete diet, exhibited but insignificant changes in the gastric muscosa. Bullock and Rohdenburg (*4*) attempted to demonstrate that Fibiger's results were caused by nonspecific chronic irritation. They resorted to the use of mechanical and chemical irritants and obtained results similar to but not identical with those of Fibiger.

Pappenheimer and Larimore (*5*) reported experiments which indicated for the first time that dietary deficiencies might be a factor in the etiology of gastric lesions. They were unable to ascertain the exact nature of the deficiency in their experimental diets. Subsequently, a number of investigations dealing with the role of dietary deficiency in gastric lesions have been reported. Detailed accounts may be found in the recent work of Klein and Palmer (*6*), Beck and Peacock (*7*), Morris and Lippincott (*8*), and Brunschwig and Rasmussen (*9*).

Since the evolution of our present knowledge of chemical carcinogens, several investigators have described experiments designed to demonstrate a relationship between dietary fats and gastric cancer. Roffo (*10*) reported the production of adenocarcinoma of the glandular portion of the stomach and "fuso-cellular sarcoma" of the liver in rats fed heated fats; Domagk (*11*) found tumors in the stomachs of mice fed a diet rich in olive oil; Waterman (*12*) claimed that he obtained malignant tumors in the stomachs of mice fed cholesteryl oleate; Veldstra (*13*) offered Δ 3,5-cholestadiene as a possible link between heated

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dietary fats and gastric neoplasia; and Widmark (*14*) concluded that extracts of roasted foods (coffee, butter, horse muscle) contain one or more carcinogenic substances. The chemistry involved in the experimental work cited was analyzed by Cook and Kennaway (*15*) and the pathology by Klein and Palmer (*6*). Recently, Steiner, Steele, and Koch (*16*) investigated the possible carcinogenicity of drastically heated meat fats, cholesterol, sesame oil, and acrolein. The feeding and subcutaneous injection of these heated substances led to essentially negative results.

Roffo (*10, 10a*) fed rats bread and milk to which was added an equal amount of lard, beef tallow, mutton tallow, or olive oil, which had been heated to 350° C. for one-half hour. Beck and Peacock (*7*) fed rats and mice similarly heated fats incorporated into an adequate diet. They were unable to find any evidence of malignant glandular tumors or of sarcomas. Papillomatosis was found in the forestomach of animals fed heated-fat diets and was attributed to an "anti-vitamin A" factor in the heated fats.

The temperature employed by Roffo was much higher than that used in ordinary cooking procedures, nevertheless, his observations appeared to be of sufficient interest to warrant further study. The hypothesis that merely heating a major dietary component resulted, presumably, in the formation in that foodstuff of substances carcinogenic, at least for the rat, raised several questions: (1) Could Roffo's claim that the heating of fat converted cholesterol, or other sterol into a carcinogenic substance be verified either chemically or biologically? (2) Could gastric neoplasms be produced with regularity by feeding rats a diet rich in heated fat? (3) Could prolonged heating of fats at temperatures lower than those used by Roffo produce the same effect on the stomach of the rat?

The present paper reports the results obtained by feeding heated lard to rats until they were dying, and the gross and microscopic examination of the stomach, liver, and certain other affected tissues. Simultaneously, a chemical study was made on lard that had been heated under varying conditions, with the purpose of clarifying the nature of the chemical changes that occur when lard is heated at extreme temperatures.

In the original plan of our work, it was tentatively proposed to study the effects of heating three animal fats (pork, beef, mutton) and one vegetable fat consisting for the most part of a partially hydrogenated cottonseed oil. The physical equipment and technical assistance for studying these four fats simultaneously was not available. Therefore, because of the extensive use of lard in the human dietary, it was decided to study lard first. An examination of Roffo's protocols indicated that his results were much the same, regardless of the fat used. Any degree of severity or difference in time of onset of the stomach lesions was not apparent in the groups of rats fed the four heated fats in his experiments. Hence, it seemed desirable to us to concentrate on just one fat and to study the effects of variations of time and temperature of heating.

MATERIALS AND METHODS

Three-month-old Wistar rats, equally divided as to sex and selected at random, were used. They were placed on the special high-fat diets after the period of most rapid growth had occurred. These rats were of the same approximate age as the animals used in the extensive experiments described by Roffo (*10*). The animals were housed individually in wire-cloth-bottom metal cages. Individual records of food intake and body weight were made at weekly intervals throughout the experiment. Daily observation was

made of each animal, and toward the end of the experiment moribund animals were killed by gas and immediately autopsied. In spite of every precaution, some animals died at night and autopsy was not performed for some hours after death. In such cases, if the animal had undergone extensive autolysis, it was not included in the interpretation of the histologic material.

The temperature of the animal room was maintained at 80° F. during the period of the year when heat was available. During a few hot days in summer the temperature rose occasionally to 88° to 90° for short periods. Since the animals were in wire-cloth-bottom cages, they did not appear to become overheated on such occasions.

The cages were washed and sterilized in boiling water followed by live steam, at frequent intervals. No nesting material was provided for the first 18 months, and during this period many animals became excessively greasy. It is believed that this condition was brought about by the inability of the rats to groom themselves properly after getting fat smeared on their paws while eating the high-fat diet. Some loss in food probably occurred in this way which could not be taken into consideration in the food-intake records. After about 18 months crepe-paper shavings were placed in all cages, and it was observed that the greasy condition of the animals' hair soon disappeared. At the same time the bedding material became oil-soaked and had to be removed frequently. This was not due to actual spillage of food but resulted from food that adhered to the feet and hair of the animals.

EXPERIMENTAL PROCEDURE

The lard was homogeneously rendered under carefully controlled conditions and was of a quality equal or superior to the best grades of market lard. It was stored in hermetically sealed cans at −10° C. After 2 years samples of this lard showed no significant changes in physical or chemical characteristics.

The lard was heated only as needed for the experimental diet. Stored lard, 3 pounds at a time, was warmed to the molten state on the water bath and poured into either a pyrex beaker or a glazed, 4-l., Coors porcelain beaker and heated on a heavy-duty hot plate. Thirty, forty-five, and sixty minutes were usually required to reach temperatures of 200°, 300°, and 350° C., respectively. After the desired temperature was reached, it was maintained within a range of ±3°. At temperatures above 330°, the rate of thermal decomposition of the lard was accelerated and fuming became severe. Preliminary lots were prone to catch fire spontaneously. In order to prevent this, the heating vessel was covered with a galvanized-sheet-iron plate, which was notched for insertion of a thermometer. This plate served to prevent contact of atmospheric oxygen with the volatile fumes at or above their flash point. After the desired temperature was maintained for the required time, the lard was allowed to cool until it could be conveniently handled (200°–250°), poured into suitable containers, and returned to the cold room. Representative samples of the heated lard were frequently set aside for immediate chemical study.

Preliminary exploratory experiments revealed that our rats were loath to eat and were unable to survive more than a few weeks on a diet containing 50 percent of lard that had been heated at 350° C. for one-half hour. Lard heated under these conditions was found to have an unpleasant taste and odor; when heated at lower temperatures it appeared to be as palatable as unheated lard. Because of the relative

paucity of information in the literature concerning the chemical changes induced in severely heated edible fats, it was necessary to study the nature of the decomposition. The results of the studies made on the unheated stock lard and on samples heated from 200° to 250° for varying periods are reported elsewhere (*18*).

The diets, which were prepared at 2-week intervals, were stored in glass vessels in a cold room maintained at −5° C. and used as needed. Fresh diet was given the animals in clean, dry, glass feeding cups once a week, and sufficient food was given to last an entire week. With the technical facilities available, it was not possible to feed the animals at more frequent intervals. All diets were isocaloric so that the food consumption should be approximately the same and comparable for all groups of animals.

The basal portion, comprising 50 percent of the complete diet, contained the following substances:

	Percent
Ground whole wheat	22.35
Skim milk powder	24.00
Brewers' yeast (debittered)	2.00
Corn oil, fortified with vitamin A ester [1] and calciferol [2]	[3] 1.00
Sodium chloride	.50
Ferric citrate	.15
Total	50.00

[1] Vitamin A ester was supplied by Distillation Products, Inc., Rochester, N. Y.
[2] Calciferol was supplied by Mead Johnson Co., Evansville, Ind.
[3] Equivalent in vitamin A and D to 8 percent U. S. P. XI cod-liver oil.

The complete diets, which consisted of the basal portion plus added lard, are listed in table 1. The protein content of the diet was roughly 11.5 percent. The wheat was a soft winter variety and therefore of low protein content. With the addition of yeast and vitamins this diet was probably of a higher nutritive quality than the bread and milk diet Roffo fed his rats. It is believed that the complete diet containing [illegible] percent [illegible]

TABLE 1.—*Diets used in experiments with heated lard*

Diet No.	Fat component	Treatment: Temperature of heating	Treatment: Time of heating	Proportion of diet
		°C.	*Minutes*	*Percent*
71	Lard	None	None	50.0
72	do	300	30	50.0
73	do	350	30	35.0
73	do	None	None	15.0
74	do	300	120	50.0
75	do	None	None	38.0
75	Free fatty acids from unheated lard	None	None	12.0

satisfactory for the adequate nutrition of the rat over an experimental period of more than 20 months. The control animals were able to increase in body weight throughout this period and showed no gross clinical evidence of nutritional deficiency.

Each group contained 30 animals equally divided as to sex, except the group on diet 75, which consisted of only 20 animals. Figure 1 gives the average body-weight curves of the female rats for the first 75 weeks of the experiment. As there was no essential difference between males and females, only 1 set of body-weight curves is presented. In the group of rats fed diet 74, which contained lard heated to 300° C. for 2 hours, the average body weight decreased for 2 weeks at the start of the experiment, after which the body weight was just maintained for about 4 months; then there was a gradual decrease in weight to the end of the experiment. In the case of the animals fed diet 73, which contained lard heated to 350° for 30 minutes, the initial loss in weight lasted for 4 weeks and amounted to approximately 20 percent of the initial body weight. The animals were able to regain little of this lost weight and appeared to follow a general downward trend in-

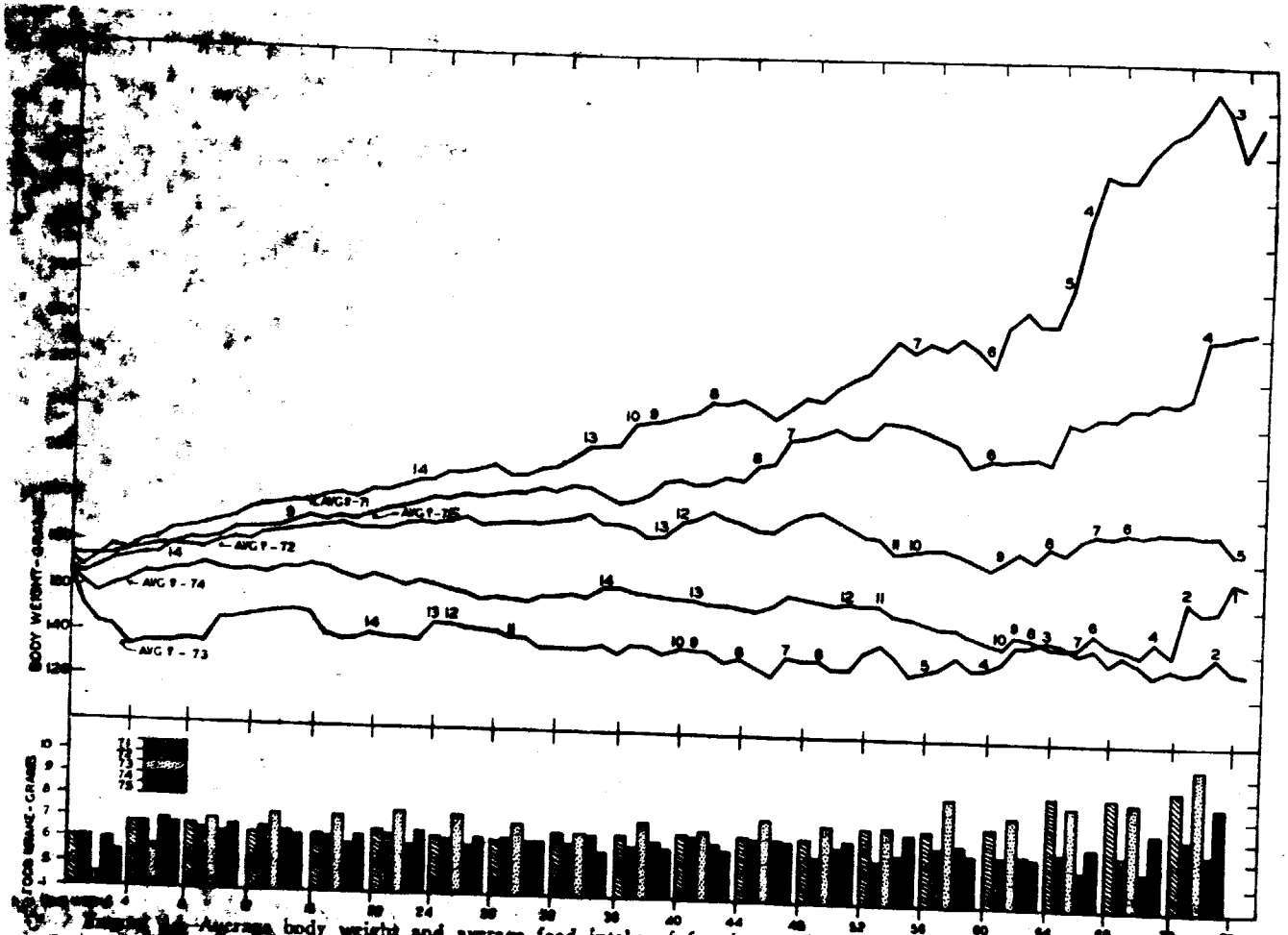


Figure 1.—Average body weight and average food intake of female rats fed diets containing 50 percent of heated and unheated lard.

except between the eighth and fourteenth weeks.

The average body weight of the group fed diet 72, which contained lard that had been heated to 300° for 30 minutes, increased gradually for 6 or 7 months, after which no additional increase in body weight was observed.

In contrast with the three groups that received the heated fat are the two receiving diets 71 and 75, respectively, which contained unheated lard. These groups gained in body weight for the entire experimental period. The group fed the diet containing 12 percent of fatty acids from unheated lard (diet 75) in place of an equal amount of lard were never quite so heavy as the control group that received the diet containing 50 percent of unheated lard.

The food-intake values for the five groups of rats are also given in figure 1. These values are calculated average daily food intakes for periods of 4 weeks per histogram. There appears to be no essential difference in food intake in any of the groups, except the group receiving diet 73 that contained lard heated to 350° C. for 30 minutes. The animals in this group appeared to be more greasy than those in the other groups. It is believed that the calculated greater food intake for this group may be owing to the greater tendency of the animals to smear the food on their paws and bodies. The diet was not only distasteful to the animals but may have been toxic also.

The figures above the body-weight curves (fig. 1) refer to the number of animals surviving at any given time. There appears to be some difference in survival of the rats fed diet 75. In this group 40 percent of the animals were still alive at the end of 75 weeks, while only 20 percent of the controls, 33 percent of the animals on diet 72, 15 percent of those on diet 73, and 7 percent of those on diet 74 were still alive at this time. After about 90 weeks, only 5 percent of the animals were still alive, except in the group fed 12 percent of free fatty acids.

An unusual type of paralysis appeared in a considerable number of the rats fed heated lard. Figure 2 shows a rat in this condition. The animal seemed to be able to lift its head with great difficulty and lay most of the time with head resting on the floor of the cage. It had very poor coordination when walking; instead of lifting its weight on its legs, the animal had a tendency to walk on its hocks, as indicated by the right hind leg (fig. 2). The condition was a progressive type of paralysis in that the first clinical evidence of the condi-

tion was a pronounced trembling movement of the head, followed by increased inability to use the legs; in some animals paralysis was so pronounced that they could not eat unless the food cup was turned over on the side. The forelegs became involved earlier than the hind legs. This paralysis had some of the characteristics of vitamin E deficiency described by Mackenzie, Mackenzie, and McCollum (*17*). The condition was never observed in rats fed the diets containing either unheated lard or unheated lard plus fatty acids therefrom. It was assumed, therefore, that the paralysis was caused by some factor in the heated fats, which possibly involved destruction of the vitamin E in the diet.

Pathology

One hundred and eleven rats were autopsied. Fifty-five of the animals were permitted to live under the experimental conditions until death and were then autopsied as soon as possible. Later in the course of the experiment, when it became apparent that an animal was about to die, it was killed so that the tissues could be obtained unaffected by autolysis. All tissues were routinely fixed in Zenker's fluid and stained with haematoxylin and eosin. In addition, liver was fixed in formalin (U. S. P. 1:10) for the sudan IV stain and in absolute alcohol for the glycogen stain.

Stomach

Gross examination of the forestomach usually disclosed no apparent lesions. However, in nine of the animals this organ presented varying numbers of discrete, white, nodular elevations of the mucosa measuring up to 3 x 2 mm. Microscopically, such elevations proved to be benign

Forestomach ulcer of rat on diet 73 for 14 months. × 95.

papillomas (fig. 3); apart from these ulcers were sometimes found. They consisted of a defect in the squamous epithelium with nonelevated margins. The base was formed by a richly vascular granulation tissue containing many leukocytes, chiefly eosinophiles. The granulation tissue often extended laterally for a considerable distance between the muscularis mucosae and circular muscular coats.

In some rats, gross examination of the glandular portion of the stomach revealed the presence of large and small discrete ulcers (fig. 4). In some instances as many as five ulcers were present in one stomach. They were usually in the prepyloric region on the lesser curvature. The largest ulcer measured 5 x 3 x 2 mm. The margins were elevated and undermined, and the base was pearly gray. In addition to these well-formed ulcers, irregular hemorrhagic erosions up to 4 mm. in diameter were seen in scattered parts of the pyloric portion of the stomach.

Microscopically, the ulcers appeared as well-defined lesions (fig. 5). In the smaller ulcers, the mucosa of the margins appeared perfectly normal, but in some instances was atrophic and fused to the base of the ulcer (fig. 6). The base of such an ulcer was formed by granulation tissue frequently extending through the muscularis mucosa laterally for some distance beneath the overlying intact epithelium (fig. 7, *A*). There was abundant exudate in this granulation tissue, chiefly polymorphonuclear leukocytes, with many eosinophiles. In some of the ulcers the base contained irregularly fragmented fibers of the muscularis mucosae and many large acini (fig. 7, *B*). The latter occurred in small groups and sometimes suggested the appearance of adenomatous

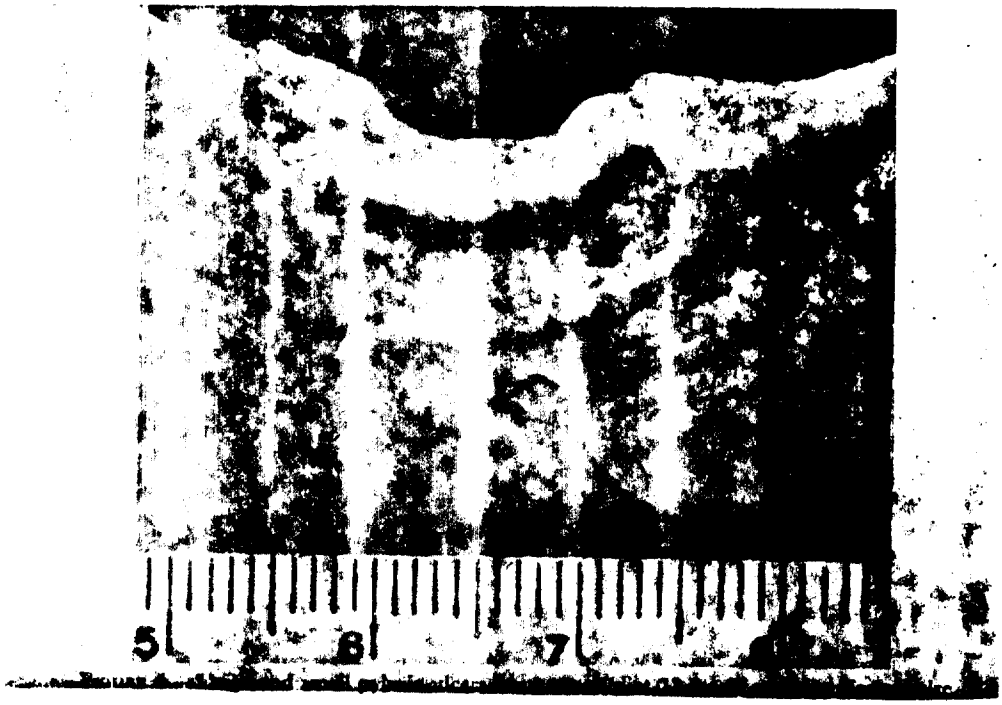


FIGURE 5.—Stomach ulcer of rat on diet 73 for 14 months. × 58.

FIGURE 6.—Margin of stomach ulcer showing atrophic epithelium. Rat was on diet 73 for 14 months. × 85.

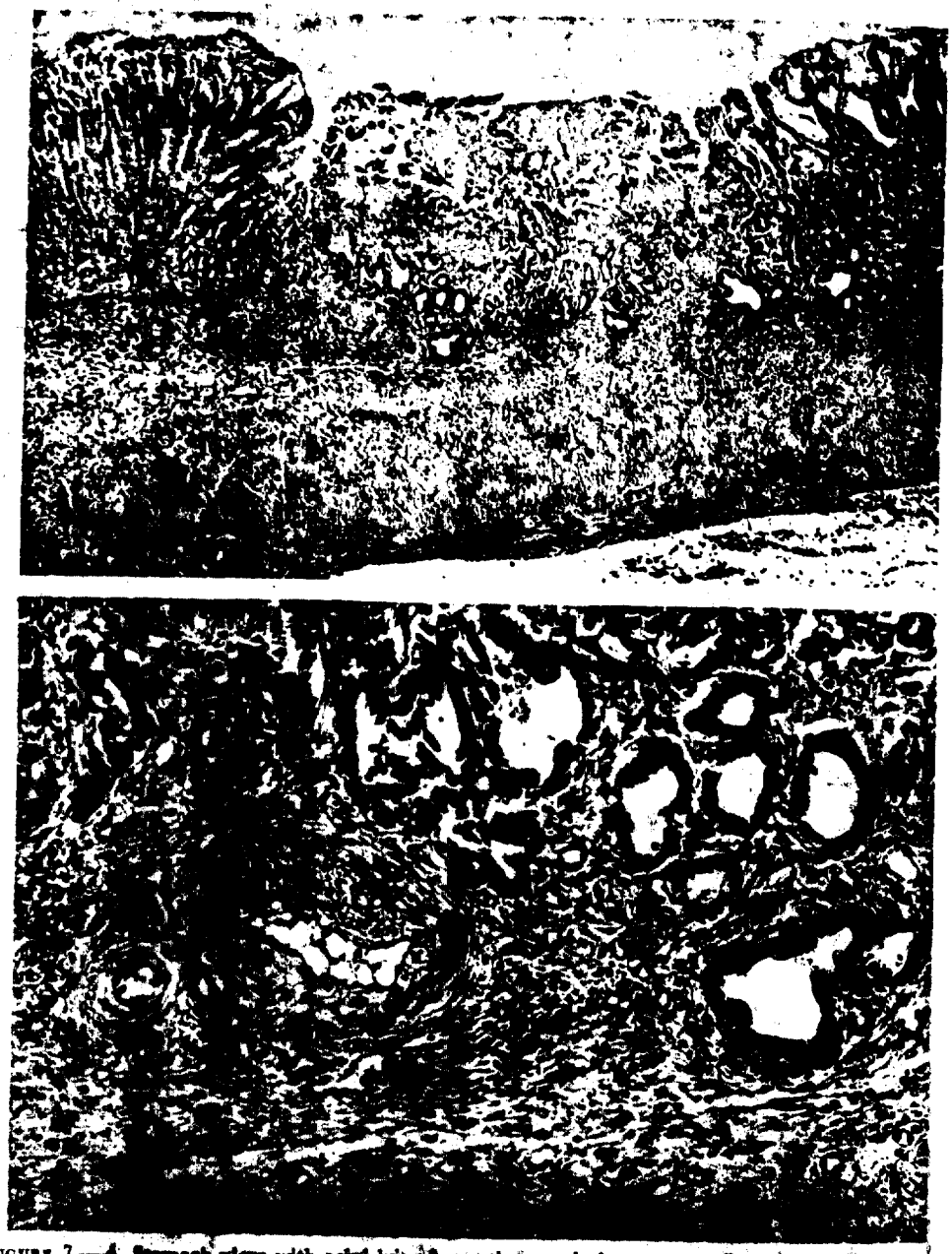


FIGURE 7.—*A*, Stomach ulcer with acini lying beneath muscularis mucosae. Rat was on diet 73 for 17 months. × 42; *B*, Higher magnification of the acini. × 190. (*A*, upper, and *B*, lower part of figure.)

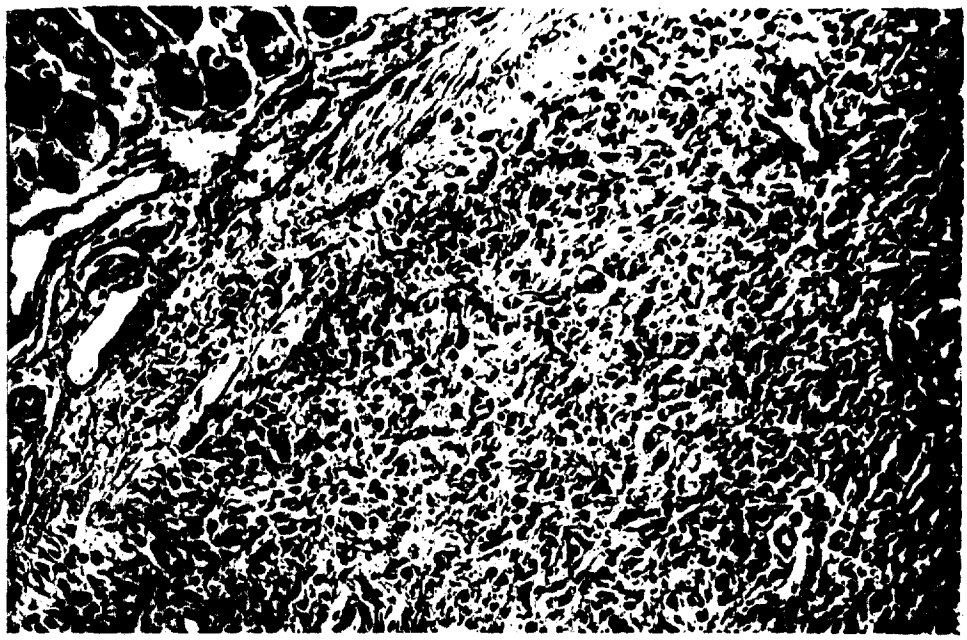


FIGURE 8.—Exuberant granulation tissue in nodulelike formation, from rat on diet 73 for 18 months. × 210.

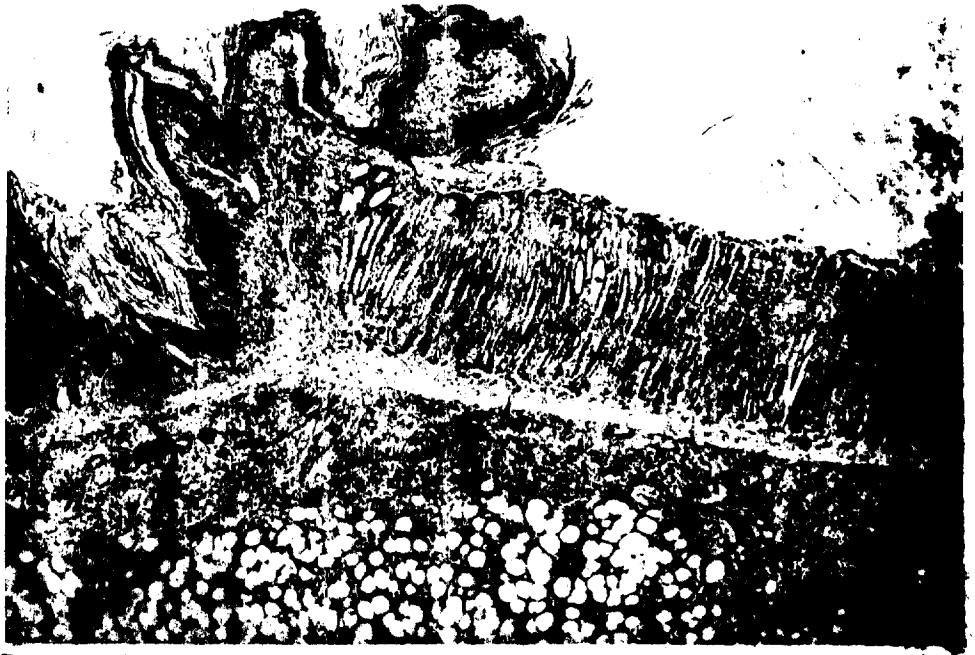


FIGURE 9.—Mucosal and submucal inflammatory granulation tissue from rat on diet 73 for 18 months. × 100.

hyperplasia. However, the acini, though dilated, were not markedly irregular. The individual cells formed single layers in orderly arrangement and were not anaplastic in character. In no instance did these acini penetrate the muscular coats. The acini were surrounded by dense granulation tissue and apparently were associated with regeneration, and an attempt on the part of the ulcer to heal. There was no indication that they represented a carcinomatous process.

Another feature observed grossly was the presence in one animal of several firm, nodular elevations, 4 x 3 x 2 mm., in the pyloric portion of the stomach. The mucosa overlying the nodules was intact in some instances and apparently ulcerated in others. Microscopically, these lesions appeared to be composed of extremely vascular granulation tissue (fig. 8). Because of the character of the lesion, consideration was given to the possibility that it might be a fibrosarcoma or possibly a sclerosing hemangio-endothelioma. However, after careful study with special stains, a diagnosis of granulation tissue was made. One of the confusing features was the occasional penetration of granulation tissue through the muscular coats into the serosa. In view of the fact that transition stages existed between this suggestively infiltrating lesion and other areas of unquestionable granulation tissue, it was felt that a diagnosis of malignancy was not justified. In early lesions, the mucosa was edematous and contained great numbers of eosinophiles (fig. 9). Such exudate was also found in the submucosa. In successive stages it was observed that fibrin was present and that subsequently fibroblasts were laid down so that the edematous tissue underwent [illegible] into a vascular fibroblastic granulation tissue. Therefore, these [illegible] considered [illegible] inflammatory processes. Often there was no evident overlying ulceration.

Liver

In the control animals, after about 1 year, the liver appeared enlarged, grossly, and of light red color with smooth contours. Miscroscopically, islands of normal parenchyma were seen, the surrounding tissue showing noticeably fatty changes (fig. 10, *A* and *B*). These changes were characterized in some instances by large fat globules appearing in the cytoplasm and crowding out the nucleus. In other instances, the fat appeared as finely divided droplets in the cytoplasm. Both types occurred in any portion of the lobule. In isolated instances there were focal areas of fibrosis (fig. 10, *C*) in these regions of fatty change, resulting in slight irregularity and retraction of Glisson's capsule. This connective tissue enclosed irregular islands of parenchyma and sometimes radiated from the portal spaces, but other than this had no characteristic distribution. This lesion was considered to be a fatty cirrhosis.

As early as 7 months after the animals were placed on diet 73, gross irregularities were observed in focal areas of the liver. These appeared as gentle, sweeping undulations which later became pitted, granular, and depressed, leaving elevated ridges in the intervening spaces. The liver was not uniformly involved, but at times as much as half of one of the large lobes, particularly at the margins and on the undersurface, showed the lesion. Microscopically, such lesions resembled the picture of acute yellow atrophy (fig. 11, *A* and *B*). The initial event appeared to be focal degeneration of hepatic cells (fig. 12, *A*), occurring in any portion of the lobule or involving several contiguous lobules. The hepatic cells were in various

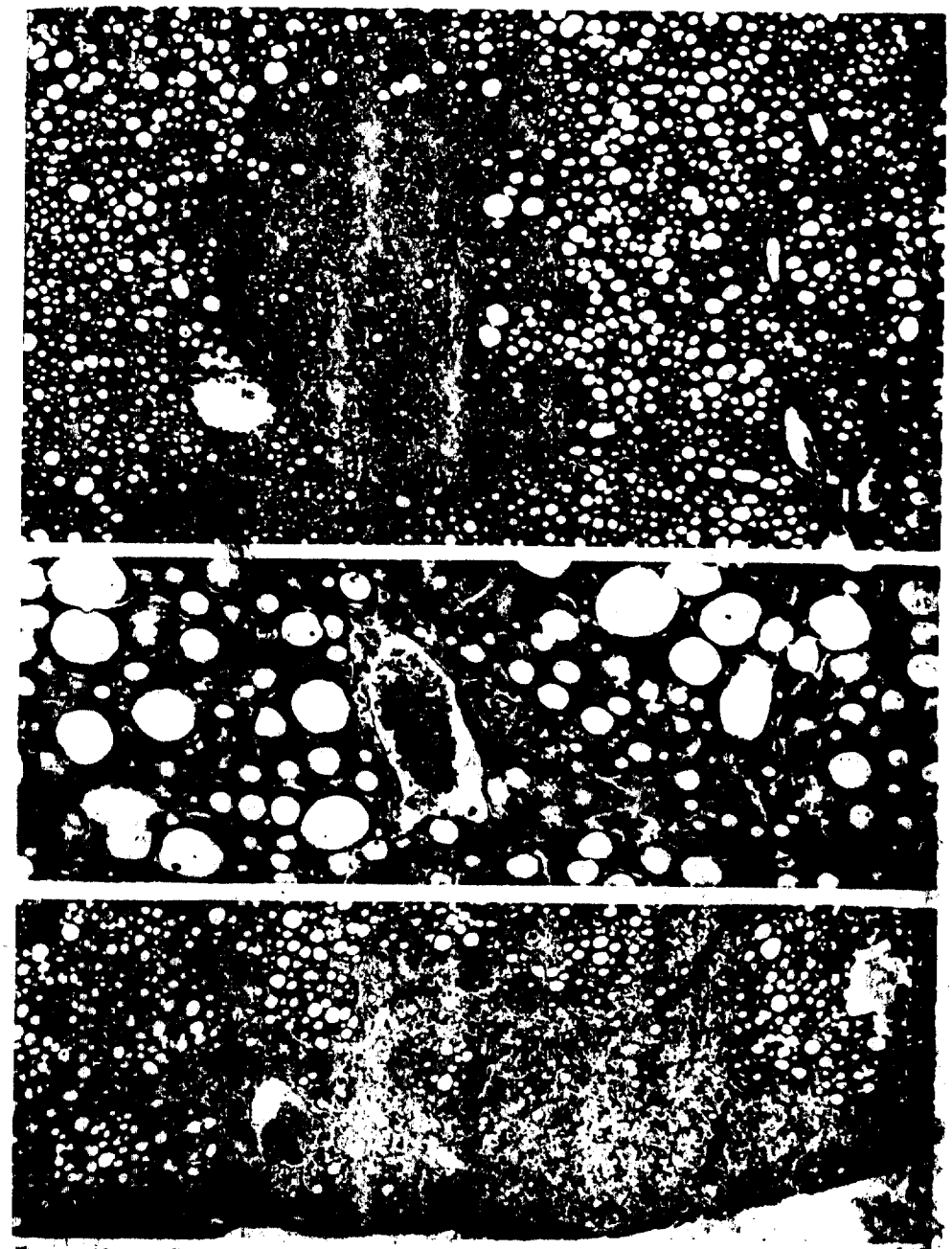


FIGURE 10.—*A*, Fatty cirrhosis of liver in rat on diet 71 for 18 months. × 58. *B*, Higher power than *A*, showing microscopic character of fatty change. × 195; *C*, Fatty cirrhosis with fibrosis of liver in rat on diet 71 for 14 months. × 64. (*A*, top; *B*, middle; and *C*, lower part of figure.)

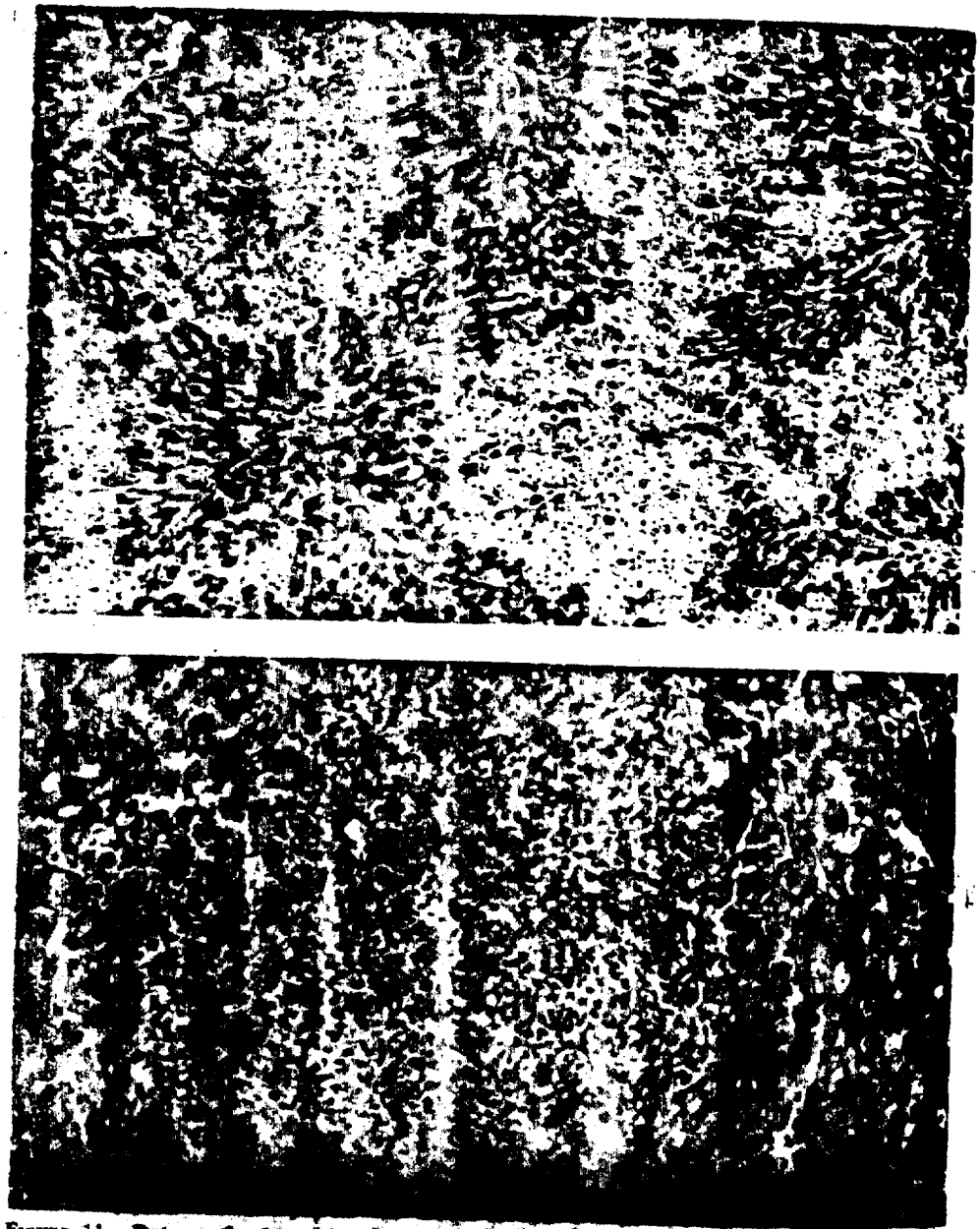


FIGURE 11.—Degenerative hepatitis. *A*, From rat on diet 73 for 9 months. × 95. *B*, From rat on diet 73 for 17 months. × 265. (*A*, upper, and *B*, lower part of figure.)

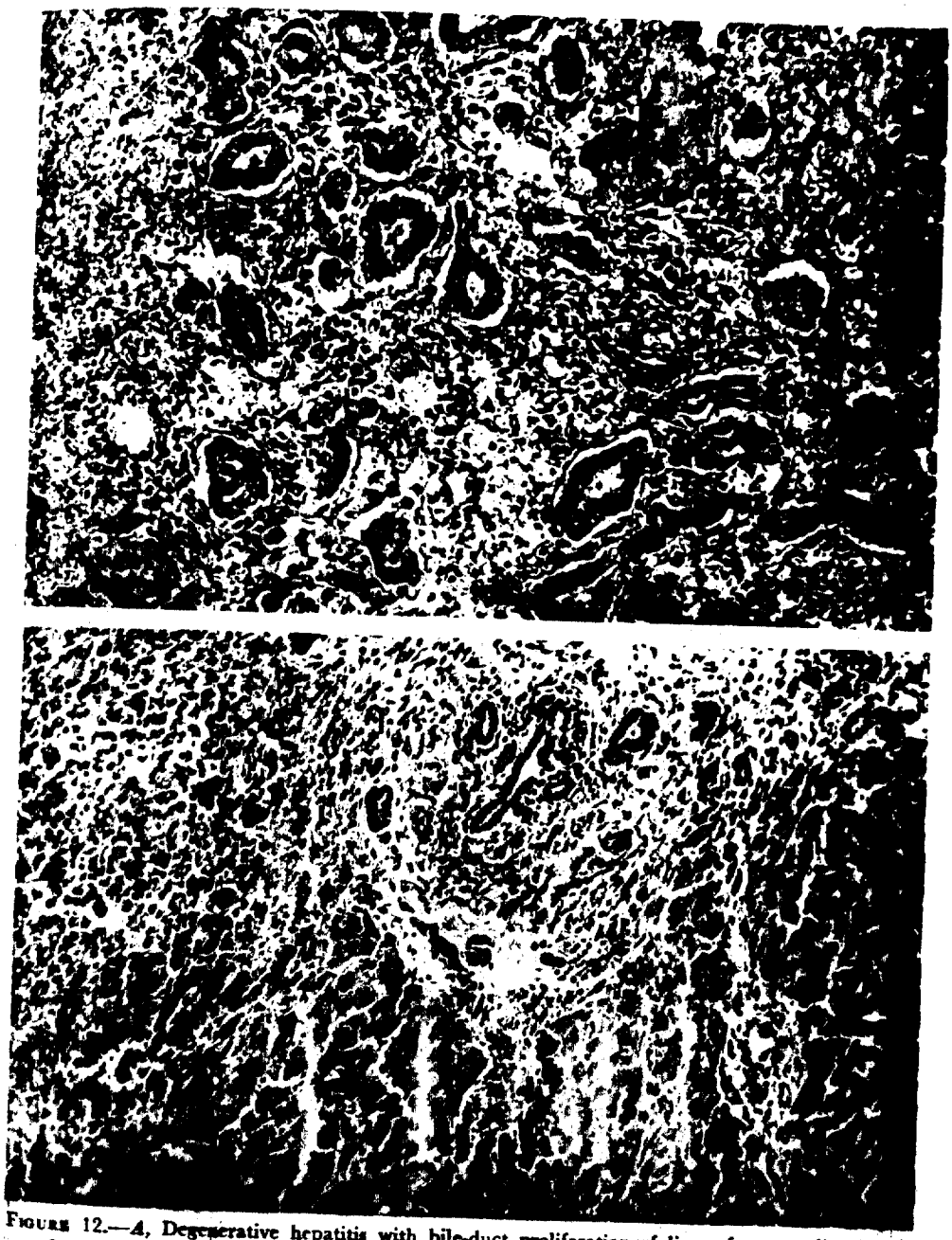


FIGURE 12.—*A*, Degenerative hepatitis with bile-duct proliferation of liver of rat on diet 73 for 7 months. × 235. *B*, Degenerative and proliferative hepatitis of liver of rat on diet 73 for 12 months. × 155. (*A*, upper, and *B*, lower part of figure.)

stages of degeneration with the pyknotic nuclei sometimes representing the only remaining cellular debris. These necrotic areas infrequently became infiltrated with polymorphonuclear leukocytes, but rarely, if ever, with any lymphocytes or plasma cells. This degenerative process frequently involved the parenchymal cells around the portal spaces, leaving the bile ducts still well-preserved. When there was sufficient collapse of parenchyma, many bile ducts appeared crowded into a small area, and sometimes there was epithelial hyperplasia with three or four layers of cells (fig. 12, *B*). Scattered through such areas was a slight proliferation of fibroblasts, sparsely strung out between bile ducts and remaining hepatic cells. This lesion was considered a degenerative hepatitis, eventuating into cirrhosis in some cases.

Heart

Grossly, no lesions were observed in the myocardium, but microscopically, there were many focal areas of myocardial degeneration and fibrosis (fig. 13, *A*). These areas appeared not unlike healing infarcts. The lesions were not related to a disturbance in the coronary vessels, however, since the latter appeared intact. Muscle fibers, individually and in groups, became necrotic and disappeared, followed by fibrosis. Inflammatory cells were not present. This lesion was considered to be focal degenerative myocardial fibrosis.

Kidney

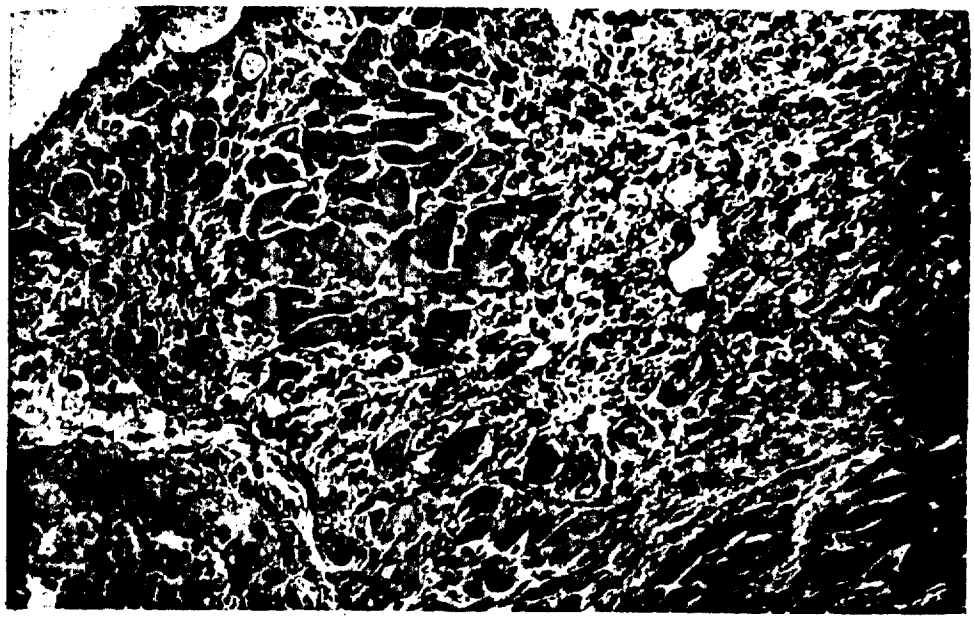
Grossly, the capsule of the kidney usually appeared smooth, but occasionally there were small retracted areas. In such areas minute cortical scars were present microscopically (fig. 13, *B*), which may not have been the result of experimental procedure, but rather the aging of the animals. These lesions were also observed in old control animals, but less frequently. In the experimental animals, the lumina of the collecting tubules were frequently filled with granular debris. The exact nature of this material was not determined. It did not contain iron or red blood cells. Often the lining cells were atrophic and showed areas containing mitotic figures. No exudate was found between adjacent tubules. This lesion appeared to be of the nature of a tubular nephrosis.

Table 2 gives the histologic changes which were observed in the liver and glandular stomach of rats on these heated fat diets. Most of the degenerative hepatitis occurred in the group fed the diet heated to the highest temperature. No degenerative hepatitis was found in the control group, and only one animal had hepatitis in the group receiving diet 75,

TABLE 2.—*Liver and stomach lesions in rats fed heated lard diets*

Diet No.	Heat treatment of lard		Liver			Glandular stomach		
	Temperature	Time	Fatty changes		Degenerative hepatitis cases	Ulcer	Carcinoma	Sarcoma
			Moderate	Marked				
		Minutes	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
71	None (control)		[illegible]	[illegible]	0	1	0	0
72	300	[illegible]	[illegible]	[illegible]	5	2	0	0
74	300	[illegible]	[illegible]	[illegible]	1	1	0	0
73	350	[illegible]	12	[illegible]	[illegible]	4	0	0
75	None; [illegible] fatty acids	[illegible]	9	2	[illegible]	0	[illegible]	0

[1] 1 animal [illegible] of the lung at [illegible] months.
[2] 1 animal [illegible] carcinoma [illegible] months old; 1 ovarian tumor and also myocardial degeneration [illegible] an animal 27 months old.



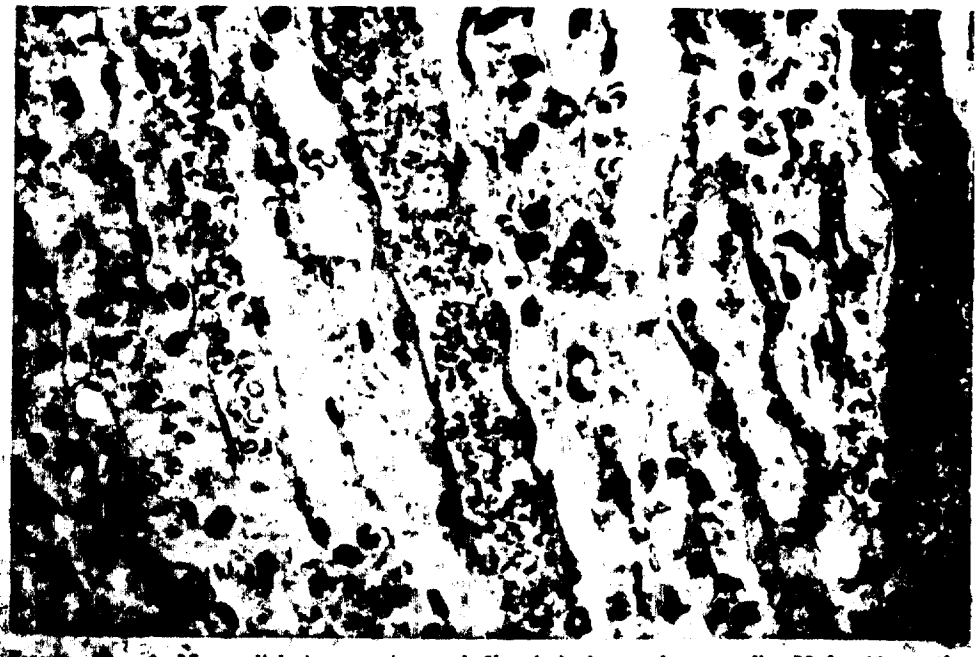


FIGURE [illegible].—*A*, Myocardial degeneration and fibrosis in heart of rat on diet 72 for 18 m[illegible] × [illegible]; *B*, Nephrosis in rat on diet 74 for 15 months. × 415. (*A*, upper, and *B*, [illegible] of figure.)

which contained unheated lard and free fatty acids from unheated lard. Half of the glandular ulcers observed were in the animals that received the diet heated to the highest temperature. One ovarian tumor and two lung neoplasms were found in rats 26 to 27 months old. These lesions were probably of spontaneous origin.

DISCUSSION

It is impossible to say at this time why the experiments described herein did not give results similar to those obtained by Roffo. There are few statistics available on stomach and liver lesions normally present in old rats. Roffo's protocols indicate that some animals lived longer than those in the present experiment. However, only about one-half of the total number of rats with which Roffo started are accounted for in his published records.

The diet Roffo used appears to have been less rich in several essential dietary constituents, as compared with the basal diet used in this work. Therefore, it is impossible to say whether or not basal-diet differences could have been responsible for the striking difference in the lesions found in the stomach and other organs. There were many animals in our groups receiving heated fat which showed very definite clinical evidence of toxicity. The chemical decomposition of heated lard observed by Larson and Morris (*18*), which appeared to be a function of the time and temperature of heating, resulted in the disruption of some double bonds. Triglycerides were rapidly disrupted above 330° C. We believe the resulting fatty acids, or their radicals, combined in part in the form of unsaponifiable aliphatic ketones. Digitonin-precipitable cholesterol disappeared in the lard heated at 350° for 30 minutes. This decomposition of the lard components by heat seems likely to have produced toxic materials. The histologic evidence of toxicity observed in the liver, heart, and kidney of many animals in the groups fed heated fat may have been due to some of these as yet unknown pyrolytic products.

Experiments have now been started to determine the type of lesion normally present in the stomachs of old rats on a stock diet. It is of interest to determine whether or not the chronic deep ulcers observed may be of frequent occurrence in old rats.

SUMMARY

Five groups of rats were fed five nutritionally adequate diets containing 50 percent of lard, for 27 months. In two diets the lard was heated to 300° C. for periods of 30 minutes and 2 hours, respectively; in another, the lard was heated to 350° for 30 minutes. The lard in the other two diets was not heated.

There was very little difference in survival between the control group and the groups receiving heated fat, at 21 months, when only about 5 percent of the original animals were still alive. In the group of rats fed 12 percent of the fatty acids of unheated lard in place of an equal amount of lard, 25 percent were still alive at 21 months.

A progressive type of paralysis having some characteristics of vitamin E deficiency in adult rats was observed in some of the animals that were fed the heated-fat diets. The paralysis first involved the forelegs and head and was accompanied by tremors; in the later stages a definite lack of co-ordination in walking was observed.

Rats maintained on a diet consisting of 50 percent of unheated lards were able to increase in body weight during the experimental period. Other rats, maintained on diets containing heated lard in the same amount, were scarcely able to maintain their body weight, and many gradually lost

weight during the entire experimental period.

Neither gastric nor hepatic neoplasms were found under the experimental conditions employed. Chronic gastric ulcers appeared in a few rats, and cirrhosis of the liver in many fed the heated-fat diets.

Lesions observed in the heart and kidney are also described.

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DIET AND THROMBUS FORMATION: QUANTITATIVE STUDIES USING AN EXTRACORPOREAL CIRCULATION IN PIGS *

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Although there has been widespread interest in the relationship of diet to atherosclerosis and its complications, much less attention has been paid to the effect of diet on thrombus formation. For the most part, exploration of this problem has been indirect; in the first place, it has been supposed that *in vitro* tests of coagulation are good measures of the coagulant activity of the blood and thrombus formation; and in the second, it has been supposed that coagulant activity *in vivo* reflects tendency to form thrombi. In a previous study (1), we attempted to circumvent the former supposition by directing our attention to platelet survival, which can be studied *in vivo* in man and in experimental animals. The ideal method of obviating the latter supposition would be to devise methods of quantitating thrombus formation in the intact animal. Semiquantitative grading systems have been used by some investigators. As yet, however, no satisfactory, precise quantitative method has been devised.

Connor and Poole (2) and Conpor (3), recognizing that movement of the blood, particularly in the arterial circulation, may be an important factor in thrombus formation, used Chandler's apparatus (4) to study the effect of various fatty acids on thrombus formation. In their experiments, citrated blood was mixed with the fatty acid for one minute. It was then recalcified in situ the apparatus, and the time for the appearance of clotting was noted.

We have described elsewhere a method for quantitating thrombus formation in an extracorporeal circulation in the pig (5). Although providing information complementary to that given by other methods, our technique has these advantages: *1*) it allows precise quantitation and *2*) continual recirculation of the blood through the body, *3*) the flow is pulsatile, and *4*) the thrombi are formed on a surface.

A number of studies suggest that diet affects blood coagulation in both acute (6, 7) and longer-term studies (1). There is evidence from animal studies (8-11) and in man from epidemiological sources (12-14) that dietary fat influences thrombosis and the complications of vascular disease. So far there have been no quantitative reports published concerning the effects of dietary manipulations on thrombus formation in flowing blood in the arterial circulation.

The value of studying the acute effects of an unfamiliar diet on thrombus formation is limited. It seems likely that such a challenge might produce qualitatively different results from one giving the experimental animals a diet to which they were metabolically and psychologically habituated. An experiment has, therefore, been undertaken in four groups of pigs. Each group was given a different diet, which was continued unchanged for at least ten months.

MATERIALS AND METHODS

Animals. Thirty two crossed Yorkshire-Landrace pigs were randomly allocated to four groups, two female and six castrated male pigs in each. Each group was given one of the four diets listed below. At the beginning of the experiment, all pigs were 4 months old. On the same day, at the tenth month of the experiment (i.e., when the pigs were 14 months old), four pigs, one chosen at random from each of the groups, were subjected to flow-chamber experiments and then killed. Each month, again one pig from each group was used. The last four

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pigs were studied when they were 20 months old (i.e., 16 months after the start of the experiment). The animals were not fed on the morning of the flow chamber experiments, their last meal having been given late in the previous afternoon.

Diets. All pigs received isocaloric diets. The control group (C) received a standard pig ration;[1] a second group (E) received the same diet with 25% of the calories replaced by uncooked egg yolk; a third group (L) received the same diet as the controls, but with 25% of the calories replaced by lard; and the last group (LC) received the same diet as the L group with cholesterol added to the lard in an amount equal to that contained in diet E (Table I). After the lard was melted, the cholesterol was added to it. The animals gained weight in uniform manner. Their mean weights at the end of the study were as follows: Group C, 540 pounds; Group L, 592 pounds; Group LC, 553 pounds; and Group E, 554 pounds.

The extracorporeal shunt. The method has been described in detail elsewhere (5). In brief, it consists of measuring the dry weight of deposit formed under standard conditions in a standard, silicone-coated, precision-built plastic flow chamber in 20 minutes. Two flow chambers of the same design and dimensions, allocated at random for each study among the four groups, were used throughout.

Coagulation tests. A blood sample for clotting tests was taken from the arterial cannula just before each flow chamber experiment. The following coagulation tests were carried out by methods which have been described (15): the whole blood clotting time in glass and in silicone-coated glass tubes, the prothrombin time, the platelet count, the plasma thromboplastin time, and the platelet adhesive index.

Serum lipids. The serum cholesterol was determined at the beginning of each experiment by a modification of the Sperry Schoenheimer method (16), and the serum phospholipid by the method of Zilversmit and Davis (17). FFA were determined by the method of Dole (18). The serum lipoproteins were determined by the method of Gofman (19) and his associates.[2]

Statistical considerations. We previously reported that the weight of deposit in the flow chamber has a lognormal distribution (20). Whole-blood clotting time is also lognormally distributed, and plasma thromboplastin time has a harmonic normal distribution. The other clotting tests are all approximately normal in distribution (15). The appropriate normalizing transformations were used before the data were processed, but mean values were retransformed into the original scale before being inserted in the tables.

Blood cholesterol and phospholipid were lognormally distributed—these findings agree with those of several other studies. FFA showed little departure from normality. Whereas the distribution of the lipoprotein fraction S_f 0 to 12 showed little skewness, there was an increase in the variance with the mean; logarithmic transformations corrected this with little change in symmetry. The values S_f 12 to 20 were left unchanged. The lipoprotein fractions S_f 20 to 100 and S_f 100 to 400 were so abnormal that nonparametric methods were used for analysis. Simple arithmetic means, however, were used as measures of central tendency.

Multiple comparisons among the groups have been made with linear contrasts (21). We had intended that all such contrasts should be orthogonal so that the levels of significance would be independent, but one animal died in each of two groups before the experiment could be completed, and some of the results for them, accordingly, were not available. The resulting departure from orthogonality has proved unimportant. It will be evi

[1] The composition of this ration was as follows: fish meal, feeding tankage, blood meal, soya-bean oil meal, corn-gluten meal, wheat shorts, calcium carbonate, bone meal, salt 1%, calcium iodate, copper carbonate, cobalt carbonate, iron carbonate, manganese sulfate, zinc oxide, alfalfa-grass meal, dehydrated alfalfa meal, riboflavin, vitamin A, D-activated plant sterol, vitamin B^{12}, stabilized animal fat, and barley oats (protein 16%, fats 3%, fiber 8%, carbohydrate 73%). Supplied by Canada Packers, Ltd., Toronto, Ont.

[2] At the Ultracentrifuge Laboratory, McGill University, Montreal, Que.

TABLE I

Diets for experimental pigs

Group*	Composition	Estimated content			
		Protein	Carbohydrate	Fat†	Cholesterol
		Total wt			
C	Hog grower ration	16	73	3	0
L	Hog grower ration, 25% of calories replaced by lard	12	68.5	11.5	0.01
E	Hog grower ration, 25% of calories replaced by egg yolk	16.1	68	7.3	0.468
LC	Hog grower ration, 25% of calories replaced by lard and an amount of cholesterol equal to that in the egg-yolk diet	12	68	11.5	0.468

* Group C, control; L, lard; E, egg yolk; LC, lard plus added cholesterol.

† Lard and cholesterol were supplied by Lever Brothers, Toronto, Ont. The cholesterol was purified cholesterol, technical grade.

TABLE II

*Diet and weight of thrombus formed with analysis of variance of weights of deposits**

	Group			
	C	L	LC	E
	mg	mg	mg	mg
Geometric mean weight of deposit	0.144 [7]	1.012 [7]	1.060 [8]	3.224 [8]

Source	Degrees of freedom	Sum of squares	Mean square	F	p
Treatments	3	6.978016	2.319339	2.63	<0.1
C vs. L, LC & E	1	5.647541	5.647541	6.40	<0.02
L vs. LC & E	1	1.224444	1.224444	1.39	<0.25
L vs. LC	1	0.000162	0.000162	0.00	<1.0
Error	26	22.943732	0.882451		
Total	29	29.901748			

* See text. Ratios take account of unequal number of experimental animals. Numbers in brackets indicate the number of animals observed. For explanation of abbreviations, see footnote to Table I.

that be made at the contrast sums of squares is [illegible] the total sum of squares among the treatments, [illegible] is [illegible] non-orthogonality would lead, if anyone [illegible] to an efficient estimate of the differences between the [illegible] treatments. The following contrasts seemed to be [illegible] appropriate: (1) control versus fat-fed animals, (2) egg versus other fat-fed, and (3) lard versus lard plus [illegible] cholesterol. This set of contrasts exhausts the treatment sums of squares, and no further contrasts can properly be done.

RESULTS

Weights of thrombi. Geometric mean weights with the analysis of variance are shown in Table II. If all the results are pooled, there is no significant difference among them. If, however, the control animals are compared with the three fat-fed groups, the difference is significant at the [illegible] level. The lard and lard-cholesterol groups showed virtually identical results; egg-fed [illegible] produced somewhat heavier deposits [illegible] in the pooled lard and lard-cholesterol groups, but this difference may easily have arisen [illegible]. For such contrasts to be legitimate, [illegible] must be decided upon before the data are [illegible]. Therefore, in spite of the biggest dif[illegible]

[illegible] obviate this the pigs were chosen at random, [illegible] to sex and length of time on the diet. [illegible] the [illegible] effects were analyzed, a complex process [illegible] two missing values, they were found to [illegible] than the error mean square. In the interests [illegible] and in order to conserve error degrees of [illegible], the results were treated as if they were obtained [illegible] experiment performed completely at random.

ference being between pigs fed egg yolk and the control diet, this contrast would not be proper.

In vitro tests of coagulation. A summary of the results is presented in Table III, including the appropriate mean values and a summary of the analyses of variance. Note that the values obtained are unimpressive, with only one of them significant at the 2% level or less, this being the platelet adhesive index for the controls versus the other groups.

Serum lipid values. A summary of the results for the serum cholesterol, phospholipid, and FFA is given in Table IV. The results do not show significant differences between FFA values. The cholesterol values are higher in the fat-fed animals than in the controls, and among the fat-fed animals, there is a conspicuous difference between the lard and lard cholesterol groups. The phospholipid values show similar but less marked differences.

TABLE III

*Diet and in vitro tests of clotting with summary of analyses of variance**

Test†	Group C	Group L	Group LC	Group E
Whole blood clotting time				
Glass tube	5.15‡ [7]	4.85‡	5.27‡	5.08‡
Silicone tube	17.70‡ [7]	17.70‡	13.80‡	14.16‡
One-stage prothrombin time, seconds	14.3 [7]	14.5	14.8	15.2
Placing thromboplastin time, seconds	10.16§	10.11§	10.31§	9.48§
Platelet count, no./mm³ × 10³	227.5	292.5	235.0	277.5
Platelet adhesive index	0.99	1.20	1.10	1.21

Test	Error: Degrees of freedom	Error: Mean square	F ratios: Treatments	C vs. L, LC & E	E vs. L & LC	L vs. LC
Whole blood clotting time						
Glass	27	0.01729	0.115	0.019	0.003	0.378
Silicone	27	0.01933	0.691	0.644	0.251	1.146
One-stage prothrombin time	[illegible]	5.5117	0.235	0.340	0.358	0.0[illegible]
Placing thromboplastin time	28	3.07696	0.433	0.099	1.153	0.015
Platelet count	28	5,866.07	1.452	1.297	0.182	2.376
Platelet adhesive index	28	0.09297	2.697	6.392	0.563	1.157

* Eight animals were observed in each experiment unless the number in brackets indicates otherwise. See footnote to Table I for explanation of abbreviations.
† Duration in minutes.
‡ Geometric mean.
§ Harmonic mean.
¶ $p < 0.02$

TABLE IV

*Diet and serum lipid with summary of analyses of variances**

Test†	Group C	L	LC	E
Cholesterol, *mg/100 ml*	113.2‡	127.4‡	221.8‡	151.0‡
Phospholipid, *mg/100 ml*	106.1‡	139.0‡	174.8‡	142.0‡
Free fatty acid, *mEq/L*	160.9 [6]	129.5 [7]	149.1 [7]	147.8 [7]

Test	Error: Degrees of freedom	Error: Mean square	F ratios: Treatments	C vs L, LC & L	F vs L & LC	L vs LC
Cholesterol	28	0.01800	7.233§	8.136§	0.646	12.918§
Phospholipid	28	0.01716	3.623‖	8.107§	0.500	2.263
Free fatty acid	23	23.277	0.474	0.704	0.116	0.571

* Footnote to Table I explains abbreviations.
† Data are mean values.
‡ Geometric mean. Eight animals were used in each experiment unless the number in brackets indicates otherwise.
§ $p < 0.01$.
‖ $p < 0.05$.

The lipoprotein results are given in Table V. For standard S_f fractions 0 to 12 and 12 to 20, there are significant differences among the treatments. The orthogonal contrasts locate these principally between the lard and lard-cholesterol groups. Little and Shanoff (22) have shown in man that the standard S_f 20 to 100 and 100 to 400 lipoprotein fractions are lognormally distributed; however, several readings in the present study were zero; thus we abandoned the classical analysis of variance in favor of the nonparametric method of Kruskal and Wallis (23). This technique shows significant differences among the treatments for both fractions. The principal components of the treatment variance must be located by inspection; it clearly lies in the high mean values for the lard-cholesterol group, and to a lesser extent in the egg-yolk values.

TABLE V

*Diet and lipoproteins with summary of analyses of variance**

Standard S_f values	Group C	L	LC	E
0–12	125.1†	137.4†	291.0†	178.2†
12–20	5.500	4.375	101.625	24.875
20–100	4.375	2.250	30.125	9.875
100–400	0.125	1.625	8.000	2.625

Standard S_f values	Error: Degrees of freedom	Error: Mean square	F ratios: Treatments	C vs L, LC & E	E vs L & LC	L vs LC
0–12	28	0.01704	12.677‡	12.304§	0.775	24.952‡
12–20	28	2211.81	7.651‡	3.943	1.907	17.104‡
20–100	28			$\chi^2 = 10.38$§		
100–400	28			$\chi^2 = 8.86$‖		

* Each value is the mean of eight experiments expressed in mg per 100 ml. Analysis of variance was performed by the nonparametric method of Kruskal and Wallis.
† Geometric mean.
‡ $p < 0.001$.
§ $p < 0.01$.
‖ $p < 0.05$.

TABLE VI

*Correlation coefficients between weight of thrombus and various blood lipids**

Correlation between log wt of thrombus and	r	t	p
Standard S_f 0–12 lipoproteins	0.122	0.548	0.6
Standard S_f 12–20 lipoproteins	0.077	0.345	0.8
Log cholesterol	0.434	2.259	0.02
Log phospholipid	0.330	1.640	0.2
Free fatty acid	0.332	1.492	0.2

* For explanation of method see text.

Correlation between serum lipid values and thrombus formation. The correlations between the weight of thrombus, and the serum cholesterol, phospholipid, FFA, standard S_f 0 to 12, and 12 to 20 lipoprotein fractions, respectively, were computed (Table VI). In each case, the covariances were computed separately for the four groups, pooled, and the variances for the two variates treated in a like fashion. The r was then computed as the pooled covariance divided by the geometric mean of the pool variances, i.e.,

$$\frac{\sum_i \sum_j (X_{ij} - \bar{X}_i)(Y_{ij} - \bar{Y}_i)}{\sqrt{\left[\sum_i \sum_j (X_{ij} - \bar{X}_i)^2\right]\left[\sum_i \sum_j (Y_{ij} - \bar{Y}_i)^2\right]}}$$

$$\text{where } \bar{Y}_i = \frac{1}{n'} \sum_j Y_{ij}, \text{ and so forth.}$$

This method involves the estimation of eight parameters, so that eight degrees of freedom are lost. In view of the small numbers involved, we felt that the non-normality of the distribution of S_f 20 to 100 and 100 to 400 precluded the use of this method for them. The serum cholesterol alone showed a significant correlation with the amount of thrombus formed.

Relationship between in vitro coagulation tests and thrombus formation. We performed covariance analyses of the weight of the deposit formed on the various clotting tests (Table VII). The pooled mean square for the regression was

TABLE VII

Regression of clotting tests on weight of deposit

Clotting test	F	p
Clotting time		
Glass	0.129	<0.9
Silicone	1.837	<0.25
Platelet count	0.950	<0.5
Platelet adhesive index	1.513	<0.25
Prothrombin time	1.736	<0.25
Plasma thromboplastin time	2.771	<0.1

tested against the pooled residual mean error. None of the results was significant.

DISCUSSION

These data show that diets rich in fat influence the amount of deposit formed in an extracorporeal circulation in pigs. The principal difference in these isocaloric diets lay in their fat content with reciprocal changes in protein and carbohydrate of the basic diet. The egg yolk diet produced the heaviest deposit; the lard and the lard with added cholesterol diets had intermediate and almost identical effects; the standard pig ration with no added fat had the least effect. The only statistically decisive difference, however, was between the values in the control group and the three groups, taken together, with added fat. To some extent, this lack of difference among the groups reflects the way the data were analyzed, more detailed comparisons among the several groups being impossible without abandoning the principle of orthogonality among them. One point worthy of note, however, is that there is nothing approaching a significant difference in the weights of deposits between the animals fed lard with added cholesterol and those fed only unenriched lard. This suggests that added dietary cholesterol as such has little influence on thrombus formation in the shunt system at least in the range used in this experiment. This experience tallies with *in vitro* work concerning the effect of cholesterol on blood coagulation (24). The pigs given the diet rich in egg yolk had three times as heavy a deposit as those on diets containing lard. Although this difference seems to be impressive, its importance is somewhat diminished because the weights of the deposits are lognormally distributed and the difference between them is not significant ($p < 0.25$). Since the scale of the experiment was small, however, and the variation in size of deposit great, this apparent difference should be subjected to further study.

Since previous work has shown changes in coagulation tests during lipemia after the ingestion of a high-fat meal (7, 20), the question must be asked whether the differences found in our study are due to prolonged dietary treatment. As all the animals were examined after fasting for 16 to 20 hours, it seems unlikely that differences were due to postprandial lipemia. We do not yet know how long a period of dietary manipulation is required to produce changes in thrombus formation. Exploring this question and also determining how long the effect of a thrombogenic diet persists after it is discontinued are important tasks. We do know, however, that animals accustomed to their respective diets tend toward thrombosis. Over the ranges studied in this experiment, the duration of the diets had no demonstrable effect on the weights of deposit formed, nor were there differences in the results between the males and the females.

There is considerable evidence that dietary fat has at least an acute effect on some *in vitro* tests of blood coagulation (6, 7, 25). The relationship between *in vitro* coagulation, endogenous coagulation, and thrombosis has been in some doubt, but we have provided evidence from studies with man involving more prolonged dietary change, notably in fat. Not only were there alterations in some *in vitro* tests of coagulation, but also platelet survival was shortened (1). The platelet is probably a more direct measure of endogenous coagulation, and certainly a factor involved in the early stage of thrombus formation.

Our findings relate more closely to the problem of thrombus formation, and in general agree with those from less direct studies. Unlike earlier studies, however, our present one does not show a significant relationship between the amount of deposit formed and the various blood-coagulation tests. This may be attributable to the small numbers involved, but it may indicate that the effect of diet on thrombus formation and blood coagulation is complex. The increase in thrombus formation seen with the lipid rich diets may be related to some factor other than change in blood coagulation. These findings again caution against

extending the conclusions of *in vitro* clotting tests to phenomena in the intact organism.

Serum cholesterol shows a significant positive correlation with the weight of deposit. Other evidence of this experiment seems to indicate that this cannot be interpreted to mean that high serum cholesterol causes thrombus formation. The diet of lard with added cholesterol differed from the lard diet only in the amount of cholesterol, which was sufficient to double the serum cholesterol, yet there was not significant difference between the two groups in the weight of deposit formed. It seems more reasonable that, for a particular diet, a high serum cholesterol and a tendency to produce an unusually heavy deposit are separate consequences of some common factor.

Although other experiments differed in method and structure, their findings (2, 3) agree with ours in suggesting that fats are important in thrombus formation and that some fats are more active in this respect than others. Several workers have shown in experiments on animals given thrombogenic diets that for thrombosis to occur, certain fats must be present (9, 26). Despite our agreement, we cannot definitely conclude that the effects we observed are entirely caused by fat. We studied rapidly flowing blood in physical, but not in physiological, isolation from the rest of the tissues. Thus, whereas the blood was influenced by the homeostatic mechanisms in the body, at the site where the thrombus was formed, there was no contribution from other vital structures. Although the silicone-coated plastic surface was foreign, it was uniformly so throughout the experiment, and there was no reason to believe that diet changed its physical properties. Thus, we can probably conclude that our results were the effects of diet on the thrombotic tendency of flowing blood.

The present experiment shows that these changes in diet produce no catastrophic effects in the tendency of blood to form thrombi, but merely augment the process seen in the control animals.

SUMMARY

Our study concerned several diets differing principally in their fat composition, some *in vitro* tests of coagulation, the tendency for thrombi to form in flowing blood, and some blood lipid fractions. Four isocaloric diets were used: 1) a low-fat diet, and three other diets with 25% of the calories in the basic diet replaced by 2) lard, 3) egg yolk, and 4) lard and as much added cholesterol as in the egg-yolk diet.

Significant differences were produced in the platelet adhesive index, which was higher in the pigs receiving added fat than in the control group. None of the other coagulation tests showed any significant differences.

Similar results were revealed by comparison of the weight of deposit formed in the extracorporeal shunts, a mean weight of deposit lower in the control group than in the other groups ($p < 0.02$), but there were no significant differences otherwise. The egg-yolk diet produced the highest mean weight of deposit. There was no difference between the mean weights of deposits for the group given lard, and that given lard plus added cholesterol, although serum cholesterol was nearly twice as high in the latter group. For a given diet, however, there is a significant correlation ($r = +0.43$, $p < 0.02$) between blood cholesterol level and weight of thrombus.

Serum cholesterol, phospholipids, and lipoprotein, with almost perfect uniformity, were highest in the lard-cholesterol group, next highest in the egg-yolk group, and least in the control group.

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A Comparative Effect of Mild Autoxidization Of Lard and Cottonseed Oil on the Nutritional Response of Weanling Rats[1]

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Abstract

Weanling rats were fed rations containing either 20 per cent lard or 20 per cent cottonseed oil. The fats were fresh or autoxidized at 50° C to peroxide values of 150 or 300 mEq./kg. When the ration contained only 12 per cent protein significant decreases were observed in growth rates, nitrogen retention, and fresh weights of livers, spleens and seminal vesicles from rats fed rancid lard rations. However, when cottonseed oil was the source of fat the only significant effect was a slight decrease in nitrogen retention. These differences were of relatively small magnitude and were not observed in preliminary studies when the rations contained 18 or 30 per cent protein or when older rats were used.

It appears that a low level of autoxidation of ration fat is sufficient to produce detectable adverse effects in young rats fed a low protein diet even when vitamins are administered separately from the rancid ration. Further, these effects are more pronounced when lard rather than cottonseed oil is the source of fat.

NUMEROUS investigations into the toxic effects of rancid fat in the diet of monogastric animals have shown that the adverse growth and physiological responses are due in part to the destruction of vitamins and in part to the toxicity of compounds formed in the fat. Barnes *et al.* (1), Greenburg and Frazer (2), and Peifer (3) have shown that rancid fats have an adverse effect on protein utilization. Barnes *et al.* (1) reported that rancid lard in the diet was less detrimental to protein utilization when the protein intake was increased. Better growth was considered to be due to the antioxidant properties of the protein. Greenburg and Frazer (2) showed that rancid fat increased the protein requirement of rats for optimum growth, and that the growth of rats was not affected by rancid fat when the diet contained 30 per cent casein.

The rations used by Greenburg and Frazer (2) contained 10 per cent oxidized soybean oil (peroxide value = 1,060 to 1,100 mEq./kg.). Fats autoxidized to this extent would be expected to contain appreciable amounts of oxidation products other than fatty hydroperoxides. Such products include carbonyl compounds and polymers, which have been shown by Kaunitz *et al.* (4) and other investigators to be highly toxic to rats.

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Peifer (3) has reported an investigation in which he used lard autoxidized to a peroxide value of 300 to 400 mEq./kg. He noted that the reduction in growth rate and changes in organ weights of rats were most evident in a ration containing 12 per cent protein. These effects disappeared when the protein level was increased to 18 per cent.

Kaunitz *et al.* (4), (5), (6), (7), (8) have reported that there is a difference in the nutritional response to and toxicity of highly autoxidized fats depending on source and chain length. They also showed that certain fresh fats afforded more protection against the toxic effects of the rancid diet than did others.

It seemed of importance and interest to determine if effects similar to these noted by investigators using highly rancid fats are also observed with mildly autoxidized fats from different sources, such as might be present in foods which are consumed by humans. Accordingly, an investigation was initiated to study nitrogen retention, growth rate and organ weights of rats as affected by the incorporation of cottonseed oil or lard, either fresh or autoxidized to peroxide values of 150 or 300 mEq./kg., in semipurified rations containing 12 per cent protein.

EXPERIMENTAL METHODS

Twenty-four weanling male albino rats were housed individually in wire-bottomed metabolism cages designed for separate collection of urine and feces. The amount of ration supplied to each rat was restricted to an amount which would be consumed by every rat in the experiment. Food consumption always exceeded the amount needed for nitrogen balance. Water was supplied ad libitum.

The animals were distributed in a manner which minimized weight differences into the following six groups: C-0, C-150, C-300, L-0, L-150, and L-300. The letters (C) and (L) refer to the fat source, cottonseed oil or lard, respectively, and the number indicates the peroxide value of the fat. Prior to feeding the test diet, a preliminary feeding period of one week on rations containing fresh fat provided for adjustment to the ration and establishment of a uniform level of feed intake (4.0 g/rat/day). As the experiment progressed, the intake per rat increased gradually to 5.5 grams per day.

The basal ration consisted of the following in per cent: casein, (vitamin-free) 12; starch, 48; dextrose, 12.6; salt mixture U.S.P. XIV, 5; cellulose, 2; fat, 10; choline chloride, 0.2; inositol, 0.15, para-aminobenzoic acid, 0.05; and nicotinic acid 0.01. The vitamins which were included in the ration have been found to be unaffected by rancid fat. Other vitamins were given orally in a capsule three times a week four hours prior to feeding. The vitamin content of each gelatin capsule is given in Table 1.

During autoxidation, air was bubbled through while the fats were kept in an 80° C water bath. When the peroxide value reached 20-40, the temperature was reduced to 50° C until the desired peroxide levels were attained. The oxidized fats were then stored in a freezer at about −20° C until used. Diets were mixed in one—kilogram quantities and stored at 4° C for not more than two weeks. During this time the peroxide value of the fat did not change more than 10 units. The energy content of the rations was determined using a Parr bomb adiabatic calorimeter. Total nitrogen was determined by the Kjeldahl method.

For the purpose of obtaining nitrogen balance data, two seven-day urine and fecal collection periods were begun on the 36th and 71st days. On the 78th day the experiment was terminated: the rats were exsanguinated while under

chloroform anesthesia. The seminal vesicles, spleen, livers, hearts, and kidneys were excised, trimmed, and weighed immediately. The small intestines were excised at the pylorus and at the ileocecal junction, opened lengthwise, washed, blotted lightly on a paper towel, and then weighed. Prior to dry weight determinations, sections of the intestine were taken for histological study.

RESULTS AND DISCUSSION

GROWTH AND NITROGEN BALANCE

Mean growth and nitrogen balance values obtained for each group of four animals are presented in Table 2. No significant differences in growth were observed among the three groups that received cottonseed oil as a source of fat. However, during the first 36 days of the trial rats fed fresh lard grew significantly better than those in the two groups fed autoxidized lard ($P < 0.05$). This trend continued until the trial was terminated on the 78th day. Lard autoxidized to a peroxide value of 150 inhibited growth as much as lard autoxidized to a peroxide value of 300. It is interesting to note that rats fed fresh lard grew significantly better than rats fed fresh cottonseed oil ($P < 0.01$) even though no appreciable differences in energy or protein content of these rations were detected.

No statistically significant differences in nitrogen retention could be detected when the balance data from each seven-day collection period were analyzed separately. However, when the data from the two collection periods were combined a significant decrease in nitrogen retention ($P \leq 0.05$) due to rancidity ($PV = 300$, either source) was observed.

ORGAN WEIGHTS

Table 3 summarizes the organ weight data obtained from the experiment. There were no significant differences in either the wet or the dry weight of the small intestines from animals of the various groups. This fails to substantiate the earlier work of Peifer (3) who did observe an increase in the wet but not the dry weight of small intestines of rats fed rancid lard rations. He also observed an increase in the hydration of the feces, which was not observed in this study. Possibly, the differences are due to the somewhat more highly oxidized rations fed by Peifer.

TABLE 1. VITAMIN CONTENT PER CAPSULE

VITAMIN	AMOUNT
Thiamine Chloride	312.0 μg
Riboflavin	625.0 μg
Pyridoxine	625.0 μg
Ca Pantothenate	1,500.0 μg
Folic Acid	375.0 μg
Biotin	6.5 μg
Cyanocobalamin	0.1 μg
Vitamin A Acetate	250.0 I.U.
Calciferol	25.0 I.U.
Alpha-Tocopherol (free)	1,750.0 μg
Alpha-Tocopherol Acetate	1,750.0 μg
Menadione	1,000.0 μg

Table 2. The Effect of Autoxidized Lard or Cottonseed Oil on Growth and Nitrogen Balance[1]

Group[2]	Body Weight		Weight Gain[3]			Combined Nitrogen Balance
	Initial day	After 7 days	36th Day	62nd Day	78th Day	Days 36-43, 71-78
	g	g	g	g	g	mg/day
C-0	59.3	61.2	20.1	37.7	59.2	38.8
C-150	63.2	64.5	20.6	36.1	56.7	36.8
C-300	62.1	64.4	22.5	37.4	56.6	31.4[4]
L-0	65.8	67.5	32.3[4]	47.2[4]	65.6[4]	37.2
L-150	62.6	64.4	22.7	38.2	60.1	36.8
L-300	58.6	62.5	23.2	39.5	58.4	34.8[4]

[1]All data were subjected to an analysis of variance. Significant differences among means were determined by the Tukey test as modified by Snedecor (9).

[2]The letters (C) and (L) refer to cottonseed oil and lard respectively; 0, 150, and 300 indicate the peroxide value of the fat.

[3]Weight after seven days was used as initial weight gain in this experiment.

[4]Significant ($p = 0.05$).

Table 3. The Effect of Rancid Fat on Organ Weights[1,2]

Group[3]		Small Intestine	Seminal Vesicles	Spleen	Liver	Heart	Kidney
	g	(Dry tissue) g	g	g	g	g	g
C-0	3.0	0.49	0.20	0.22	4.05	0.44	1.03
C-150	2.9	0.48	0.22	0.21	4.09	0.42	0.95
C-300	3.2	0.55	0.18	0.18	4.05	0.42	0.98
L-0	3.2	0.47	0.26	0.22	4.00	0.45	0.96
L-150	3.2	0.52	0.16[4]	0.22[4]	3.70	0.44	1.06
L-300	3.2	0.44	0.15[4]	0.17[5]	3.28[4]	0.41	0.97

[1]Organ weights are expressed in terms of wet tissue. Each value is the mean of four observations.

[2]All data were subjected to an analysis of variance. Significant differences among means were determined by the Tukey test as modified by Snedecor (9).

[3]The letters (C) and (L) refer to cottonseed oil and lard, respectively; 0, 150, and 300 indicate the peroxide value of the fat.

[4]Significant ($p = 0.05$).

[5]Highly significant ($p = 0.01$).

No significant differences were found in the weights of livers, spleens, or seminal vesicles from animals fed fresh or autoxidized cottonseed oil. However, significant decreases in the weights of livers ($P < 0.05$), of spleens ($P < 0.01$) and of seminal vesicles ($P < 0.05$) were observed in the autoxidized treatments when lard was the source of fat. The diets did not significantly affect heart or kidney weights.

It is apparent that even the statistically significant differences are of rather small magnitude when the lard is autoxidized only to the limited extent employed in these investigations. Preliminary trials of a similar design conducted in this laboratory with older rats or with rations containing 18 or 30 per cent protein failed to show any significant differences among the parameters measured.

Histological observations were made on a limited number of sections taken from the small intestines of the rats. Only minor differences in the structure of the intestinal wall were noted between groups, but slight necrosis and catarrhal inflammation were noted in some animals in the rancid fat groups.

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Specifications of Fatty Acid Composition for Identification of Fats and Oils by Gas Liquid Chromatography

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The Codex Committee on Fats and Oils of the Joint Food and Agricultural Organization of the United Nations and the World Health Organization (FAO/WHO), Codex Alimentarius Commission, has for several years had under consideration a complete revision of their standards for the identification of fats and oils. As early as 1966, the Canadian Delegation proposed that the classical method be replaced by an alternate system based on the fatty acid composition of each fat or oil as determined from gas liquid chromatographic patterns. The classical method is based on the identification of a specific fat or oil by means of a combination of its iodine value, relative density, refractive index and saponification value.

Prior to the third meeting of the Fats and Oil Committee held in London April 24-28, 1967, the Canadian Delegation submitted a proposal for the replacement of the classical method with a gas liquid chromatographic (GLC) procedure. In this proposal the Canadian Delegation discussed disadvantages of the classical system, particularly the requirement for a heavy investment of analytical time and skill in many techniques, and inability, even when all four indices are considered, to resolve several admixtures containing common oils of commerce. At the same time, advantages of the gas liquid chromatographic procedure were emphasized, including the fact that it permits identification of oils which cannot be identified by the classical method and offers promise of resolving many additional pairs of oils in admixture. Furthermore, since it requires only one analysis, it can be made quite rapidly and applies equally well to refined and unrefined oils, necessitating only one set of standards.

From the beginning, at least as early as 1966, the United States supported the Canadian proposal in principle and recommended specifically that the Codex Committee on Fats and Oils urge the Codex Committee on Methods of Analysis to evaluate the current status of the science of gas liquid chromatography and, if feasible, to adopt GLC procedures that can be used by the Codex Committee on Fats and Oils.

At the fourth meeting of the Codex Committee on Fats and Oils, sponsored by the Joint FAO/WHO Codex Alimentarius Commission and held in London, April 24-28, 1967, the Canadian proposal was presented. The U.S. position on the proposal was one of partial support. This position was explained: "Full support of the Canadian proposal would not be justified, since most available evidence indicates that adoption of the proposal at this time would be premature and would lead to serious practical difficulties. Full opposition to the proposal would place the United States in a position of being ultra-conservative and unwilling to recognize the most modern scientific advances in the field of fat and oil analysis. The U.S. delegation therefore took the position that a system of identification based on GLC, in addition to the classical system, be carried in the draft standards at this stage. This would be done with the expectation that before the promulgation of the standards, the science of GLC would have progressed to a point where the present uncertainties concerning it would be eliminated and that it would provide a far more accurate means of identification than is possible with the present classical procedures. If this expectation should be realized, the classical system could then be dropped before final promulgation. The U.S. delegation argued that acceptance of its views would encourage the necessary international cooperation needed in the further perfection of the GLC system of oil and fat identification." After considerable discussion this U.S. position prevailed among the delegates from the 21 nations represented at this fourth meeting.

In comments on the Canadian proposal, submitted to delegates of the countries to be represented prior to the April 1967 Meeting, the United States delegation had presented its position, as given above. With their comments the delegation commented on the requirement for GLC standards and proposed to solicit recommendations concerning such standards from the Codex Committee on Methods of Analysis which would likely be based on the work of the International Union of Pure and Applied Chemistry. In these remarks they commented that the American Oil Chemists' Society, through its Instrumental Techniques Committee and the Gas Chromatography Subcommittee thereof, was currently very active in this field and "could probably render valuable assistance to the Codex Committee on Methods of Analysis" hopefully prior to the April 1967 meeting.

The hope for accomplishment of this objective prior to the April 1967 meeting was apparently not realized, but shortly after acceptance of its proposal at this meeting, the United States delegation, through its Chairman Lawrence Zeleny, contacted the Society with a request for assistance in the preparation of such standards. The AOCS Executive Director, C. H. Hauber, arranged a meeting which was held during the joint AACC-AOCS 59th Annual Con

(Continued on page 195A)

[1] So. Utiliz. Res. Dev. Div., USDA.
[2] E. Utiliz. Res. Dev. Div., USDA.

TABLE I

Arachis Oil[a,b]

Acids	Carbon atoms	Range
Saturated	Less than 14	<0.5
Myristic	14:0	<1.0
Palmitic	16:0	6.0–14
Stearic	18:0	2.0– 6.5
Arachidic	20:0	1.0– 2.0
Behenic	22:0	2.0– 4.0
Lignoceric	24:0	1.0– 2.0
Palmitoleic	16:1	<1.0
Oleic	18:1	40 – 72
Linoleic	18:2	13 – 38
Linolenic	18:3	<0.50
Eicosenoic	20:1	0.5– 1.5

[a] Synonyms: peanut, groundnut
[b] Fatty acid composition by gas chromatography (wt % of methyl esters).

TABLE II

Cottonseed Oil[a,b]

Acids	Carbon atoms	Range
Saturated	Less than 14	<0.5
Myristic	14:0	0.5– 2.0
Palmitic	16:0	17 –29
Stearic	18:0	1.0– 4.0
Arachidic	20:0	<0.5
Behenic	22:0	
Lignoceric	24:0	
Palmitoleic	16:1	<1.5
Oleic	18:1	13 – 44
Linoleic	18:2	33 – 58
Linolenic	18:3	<0.5
Eicosenoic	20:1	<0.5

[a] Synonyms: none.
[b] Fatty acid composition by gas chromatography (wt % of methyl esters).

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(Continued from page 186A)

TABLE III
Maize Oil[a,b]

Acids	Carbon atoms	Range
Saturated	Less than 14	<0.5
Myristic	14:0	<1.0
Palmitic	16:0	8 –19
Stearic	18:0	0.5– 4.0
Arachidic	20:0	<1.0
Behenic	22:0	
Lignoceric	24:0	
Palmitoleic	16:1	<1.0
Oleic	18:1	19 –50
Linoleic	18:2	34 –62
Linolenic	18:3	<1.0
Eicosenoic	20:1	<0.5

[a] Synonyms: corn.
[b] Fatty acid composition by gas chromatography (wt % of methyl esters).

TABLE IV
Mustardseed Oil[a,b]

Acids	Carbon atoms	Range
Saturated	Less than 14	<0.5
Myristic	14:0	<1.0
Palmitic	16:0	0.5– 4.5
Stearic	18:0	0.5– 3.0
Arachidic	20:0	<1.5
Behenic	22:0	0.2– 2.5
Lignoceric	24:0	<0.5
Palmitoleic	16:1	<1.0
Oleic	18:1	8.0–23.0
Linoleic	18:2	10.0–24.0
Linolenic	18:3	6.0–18.0
Eicosenoic	20:1	4.0–13.0
Erucic	22:1	22.0–49.0
Docosadienoic	22:2	<1.0
Tetracosenoic	24:1	0.5– 2.5

[a] Synonyms: none.
[b] Fatty acid composition by gas chromatography (wt % of methyl esters).

TABLE V
Rapeseed Oil[a,b]

Acids	Carbon atoms	Range
Saturated	Less than 14	<0.5
Myristic	14:0	<1.0
Palmitic	16:0	0.5– 5.0
Stearic	18:0	0.5– 3.0
Arachidic	20:0	<1.5
Behenic	22:0	<1.5
Lignoceric	24:0	<2.0
Palmitoleic	16:1	<1.0
Oleic	18:1	9.0–25
Linoleic	18:2	11 –25
Linolenic	18:3	5.0–12
Eicosenoic	20:1	5.0–15
Erucic	22:1	30 –60
Docosadienoic	22:2	<1.0

[a] Synonyms: colza, ravison, sarson, toria, turnip rape.
[b] Fatty acid composition by gas chromatography (wt % of methyl esters).

TABLE VI
Safflower Seed Oil[a,b]

Acids	Carbon atoms	Range
Saturated	Less than 14	<0.5
Myristic	14:0	<1.0
Palmitic	16:0	2.0–10
Stearic	18:0	1.0– 6.0
Arachidic	20:0	<1.0
Behenic	22:0	<1.0
Lignoceric	24:0	
Palmitoleic	16:1	<0.5
Oleic	18:1	7.0–40
Linoleic	18:2	55 –80
Linolenic	18:3	<1.0
Eicosenoic	20:1	<0.5

[a] Synonyms: carthamus, dundea.
[b] Fatty acid composition by gas chromatography (wt % of methyl esters).

vention in the Washington-Hilton Hotel, Washington, D.C., Tuesday, April 2, 1968. The minutes of this meeting show that it was attended by five United States or Canadian delegates or advisors to the delegates to the Codex Committee on Fats and Oils, by four members of the American Oil Chemists' Society, and by two members from American industrial organizations.

At this meeting the requirements of the Codex Committee were set forth by the U.S. Delegates. This delegation had felt in the past that the proposal to use GLC in Codex standards had been premature because of insufficient knowledge of the ranges in fatty acid composition characteristics of some of the fats and oils. However recent developments in this field had led them to the opinion that Codex standards should utilize GLC analysis at least on an optional basis, otherwise standards based entirely on the classical fat and oil characteristics would soon become obsolete.

It was emphasized by the AOCS members that the major problem in establishing such standards is the need for a comprehensive survey of the variability in fatty acid distribution for each product for which standards are to be developed in order to establish completely valid and at the same time reasonable minimum and maximum requirements for such standards. It was agreed that the Codex delegation would furnish a list of the fats and oils for which standards were now required and that the AOCS, through its Instrumental Techniques Committee, would initiate the necessary survey to establish the minimum and maximum limits for each fatty acid in each specific fat or oil.

It was also agreed that in establishing these standards, the range for each fatty acid in each fat or oil would be broad enough to include all products ordinarily encountered in world trade, the complete range expected for commercial fats or oils, but not necessarily broad enough to include all products which might be encountered under exceptional conditions. Exceptional oils, it was pointed out, can result from agronomic or genetic advances to impart special properties to a specific oil. Three examples are given to illustrate such exceptional oils (where the minimum or maximum concentration or both of one or more acids might be outside of the normal range for oils of commerce.)

1. Safflower oil can be of high linoleic or high oleic variety. In the former, the 18:2 acid would be 55% to 80% and 18:1 7.0% to 40%; in the latter, the 18:2 would be 7.0% to 40% and 18:1 55% to 80%. To cover both, 18:1 would be 7.0% to 80% and 18:2 also 7.0% to 80%.
2. A rapeseed oil has now been developed which contains no erucic acid (22:1) so if this unusual variety was included, this component would be 0% to 60%, 22:1 instead of 50% to 60%.
3. The fatty acid composition for arachis oil has been found to be "unusual" in the following cited examples: Arachidic was found to be less than 1% in eight of 11 varieties studied (1). Lignoceric was found to be less than 1% in one out of three samples investigated by Craig and Murthy; for one out of seven varieties (2), and one out of 22 samples (3).

The delegates to the Codex Committee on Fats and Oils requested specifications for the following fats and oils: arachis oil (peanut oil) (groundnut oil), cottonseed oil, maize oil (corn oil), rapeseed oil, safflower oil, sesame seed oil, soya bean oil (soybean oil), sunflower seed oil, lard (rendered pork fat), and premier jus (edible tallow). Mustard seed oil was added to this list after the fifth meeting of the Codex Committee on Fats and Oils in London, September 16–20, 1968.

The Chairman of the Gas Chromatography Subcommittee of the Instrumental Techniques Committee prepared a list, from an exhaustive search of the literature, of the minimum-maximum range of fatty acid composition, as obtained by gas chromatography. These values were submitted to several

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TABLE VII
Sesame Seed Oil[a,b]

Acids	Carbon atoms	Range
Saturated	Less than 14	<0.5
Myristic	14:0	<0.5
Palmitic	16:0	7.0–12
Stearic	18:0	3.5– 6.0
Arachidic	20:0	<1.0
Behenic	22:0	<1.0
Lignoceric	24:0	....
Palmitoleic	16:1	<0.5
Oleic	18:1	35 –50
Linoleic	18:2	35 –50
Linolenic	18:3	<1.0
Eicosenoic	20:1	<0.5

[a] Synonym: gingelly.
[b] Fatty acid composition by gas chromatography (wt % of methyl esters).

TABLE VIII
Soya Bean Oil[a,b]

Acids	Carbon atoms	Range
Saturated	Less than 14	<0.5
Myristic	14:0	<0.5
Palmitic	16:0	7.0–12
Stearic	18:0	2.0– 5.5
Arachidic	20:0	<1.0
Behenic	22:0	<0.5
Lignoceric	24:0	....
Palmitoleic	16:1	<0.5
Oleic	18:1	20 –50
Linoleic	18:2	35 –60
Linolenic	18:3	2.0–13
Eicosenoic	20:1	<1.0

[a] Synonym: soybean.
[b] Fatty acid composition by gas chromatography (wt % of methyl esters).

TABLE IX
Sunflower Seed Oil[a,b]

Acids	Carbon atoms	Range
Saturated	Less than 14	<0.5
Myristic	14:0	<0.5
Palmitic	16:0	4.0– 9.0
Stearic	18:0	1.0– 6.0
Arachidic	20:0	<1.0
Behenic	22:0	<1.0
Lignoceric	24:0	....
Palmitoleic	16:1	<0.5
Oleic	18:1	14 –70
Linoleic	18:2	20 –75
Linolenic	18:3	<0.5
Eicosenoic	20:1	<0.5

[a] Synonym: none.
[b] Fatty acid composition by gas chromatography (wt % of methyl esters).

TABLE X
Lard (and Rendered Pork Fat)[a,b]

Acids	Carbon atoms	Range
Saturated	Less than 14	<1.0
Myristic	14:0	0.5– 2.5
Palmitic	16:0	20 –32
Stearic	18:0	5.0–18
Arachidic	20:0	<1.0
Behenic	22:0	<0.5
Lignoceric	24:0	....
Palmitoleic	16:1	2.0– 4.0
Oleic	18:1	35 –60
Linoleic	18:2	3.0–15
Linolenic	18:3	<2.0
Eicosenoic	20:1	<1.0

[a] Synonym: none.
[b] Fatty acid composition by gas chromatography (wt % of methyl esters).

TABLE XI
Premier Jus (and Edible Tallow)[a,b]

Acids	Carbon atoms	Range
Saturated	Less than 14	<1.0
Myristic	14:0	1.0– 8.0
Palmitic	16:0	23 –37
Stearic	18:0	6.0–20
Arachidic	20:0	<1.0
Behenic	22:0	....
Lignoceric	24:0	....
Palmitoleic	16:1	1.5– 6.0
Oleic	18:1	36 –50
Linoleic	18:2	0.5– 5.0
Linolenic	18:3	<1.0
Eicosenoic	20:1	<1.0

[a] Synonyms: none.
[b] Fatty acid composition by gas chromatography (wt % of methyl esters)

members of AOCS knowledgeable in fatty acid composition of fats and oils and from their remarks, comments and suggestions final ranges were selected as GLC specifications.

These specifications were submitted to the U.S. Delegation to the Codex Committee on Fats and Oils, through their Chairman, Dr. Zeleny, and presented at the fifth session of the Codex Committee on Fats and Oils, sponsored by the Joint FAO/WHO Codex Alimentarius Commission and held in London September 16–20, 1968. The specifications were, according to the minutes of this meeting, well received. However, as the delegates had not had an opportunity to study them, the United States proposal ". . . that all of the Codex standards for specified kinds of fats and oils contain two alternative sets of identification requirements, one based on the classical physical and chemical characteristics and the other on fatty acids composition as determined by GLC," while supported by a majority of the delegates, was referred by the Chairman of the Fifth Session back to the delegates with the request for ". . . all participating countries to study the U.S. proposal further and to consider at the next meeting of the Committee whether GLC procedures for identifying fats and oils should be inserted into the standards as 'advisory criteria.'"

At this Fifth Session of the Codex Committee on Fats and Oils, standards for mustard seed oil were discussed. One outcome of this discussion was a request to the AOCS for inclusion of standards for this oilseed, from GLC measurements. These were subsequently furnished to M. H. Neustadt, who succeeded Lawrence Zeleny as Chairman of the U.S. Delegation.

In the meantime considerable interest has been made evident by several members of AOCS, usually with requests for the GLC specifications. It appears that the most suitable medium to make the specifications available to any interested member of the Society, while they are being considered by the Codex Committee on Fats and Oils, would be by their publication in the *Journal*.

Complete specifications for the 11 oilseeds thus far considered, including mustard seed oil, are given in the attached Tables.

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MULTIPLE MESENTERIC SARCOMATA IN RATS FOLLOWING INGESTION OF HEATED LARD.

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ROFFO (1938, 1939) has described a variety of tumours of the alimentary canal and its adnexa in rats fed on a diet of bread and milk, to which various fats heated to 350° C. were added. Among 200 rats so fed, 5 developed intraperitoneal and mesenteric tumours, associated with sarcoma of the liver; in over 1000 control animals kept during the same period no such lesions were observed.

The present report deals with mesenteric tumours which occurred in the course of experiments designed to test Roffo's views on the carcinogenicity of heated fats. Altogether, among 71 of our rats which survived more than 300 days, 4 died with multiple mesenteric tumours. The gastric lesions which occurred in our earlier experiments have already been described (Beck and Peacock, 1941).

* Working under a full-time grant from the British Empire Cancer Campaign.

EXPERIMENTAL RESULTS.

Groups 1–4 consisted of male Norwegian hooded rats, which were fed on an adequate diet of rat-cake (Beck and Peacock, 1941), plus greens once a week, and 20 c.c. milk and 0·5 c.c. cod liver oil per rat per week.

Group 1.—Lard heated at 350° C. for one hour was added to the diet of 50 rats; the average weekly consumption was about 5·7 g. per rat. Among 40 rats surviving 372 days or more, 2 died with multiple mesenteric sarcomata, 1 on the 372nd and the other on the 426th day. In the first case there was no obvious primary tumour. Multiple tumours infiltrated the intestine and spleen. Histologically the tumours were reticulo-endotheliomata. The second rat had a tumour in the liver as well as multiple mesenteric nodules, and deposits in the thymus and in the connective tissue capsule of the left suprarenal gland. Histologically the tumours were spindle-cell sarcomata. Neither of these rats was infested with *Cysticercus fasciolaris*, but three other rats in this group were so infested, but had no associated tumour.

Group 2.—Lard heated at 220° C. for 12 hours was added to the diet of 36 rats. The average consumption was about the same as in Group 1. Of 18 rats surviving 367 days, one died on this day with multiple mesenteric sarcomata. There was no obvious primary tumour in this case; the histology was that of an anaplastic spindle-cell sarcoma.

Group 3.—Lard heated at 220° C. for four hours was added to the diet of 50 rats. Two rats in this group were infested with cysticerci; one of these had a tape-worm in the jejunum and a single mesenteric tumour about 1 cm. in diameter in the corresponding segment of mesentery. This rat died 537 days after the start of the experiment. The tumour was attributed to the presence of the tape-worm.

Group 4.—For control purposes unheated lard was added to the diet of 16 rats. They were only allowed to consume about the same amount of fat as those in the experimental groups, but would have taken more. One rat in this group was infested with *Cysticercus fasciolaris*. No tumours occurred in this group.

Groups 5–7 consisted of male Wistar rats which were fed on a restricted diet of white bread (*ad lib.*) plus 30 c.c. milk per rat per week. (After 344 days peeled boiled potatoes were substituted for white bread, which was no longer obtainable.)

Group 5.—Lard heated at 350° C. for 1 hour was added to the diet of 20 rats. Of 13 rats surviving 301 days, one died on that day with multiple mesenteric sarcomata not associated with cysticercus infestation. No obvious primary tumour was found; death was due to peritonitis attributable to a perforation of the stomach by sarcomatous invasion. The nodules showed the histology of spindle-cell sarcoma. Two rats in this group were infested with *Cysticercus fasciolaris*; one of them died on the 365th day with a liver sarcoma associated with a cysticercus; there were no metastases. Four other rats developed multiple ulcerated papillomata in the fore-stomach, discovered *post mortem* between the 276th and 432nd days of the experiment.

Group 6.—Lard heated at 220° C. for 12 hours was added to the diet of 20 rats. Two rats in this group were infested with cysticerci, and in one of them, which died on the 434th day, a sarcoma in the liver enclosed the cysticercus. This tumour was a spindle-cell sarcoma. Secondary deposits were found in the diaphragm, omentum, and mesentery. The same rat and also another free from infestation, which died on the 268th day, had ulcerated papillomata of the fore-stomach.

Group 7.—Unheated lard was added to the diet of 8 rats, as controls. One of these rats was infested with cysticercus, but showed no tumour.

Thus, altogether three cases of multiple mesenteric sarcomata occurred in the 53 uninfested rats fed with lard heated at 350° C., and one in 18 surviving rats fed with lard

heated at 220° C. No such tumours occurred in the controls. Only one case of multiple mesenteric sarcomata of apparently spontaneous origin was observed by us in a series of 300 *post mortem* examinations of rats, not infested with tape worms, in other experiments not connected with heated fats.

DISCUSSION.

In discussing multiple intraperitoneal tumours in rats, it is necessary to exclude infestation with *Cysticercus fasciolaris* before attributing such tumours to any other factor. The association between this parasite and the induction of tumours in rats was first reported by Borrel (1906), and was later confirmed experimentally by Bullock and Curtis (1924, 1926). Cysticerci in the liver frequently give rise to spindle-cell sarcomata in rats but not in mice, though the parasite is the same in both species. This curious fact is the subject of further experimental study. Such tumours have occurred sporadically in our experiments. They may give rise to widespread intraperitoneal metastases, in which, of course, the association with the parasite is not obvious. Indeed, it is necessary to incise all liver tumours in rats before *Cysticercus fasciolaris* infestation can be excluded. Roffo (1938, 1939) has described multiple intraperitoneal tumours, apparently secondary to large liver tumours, in rats fed with irradiated cholesterol or with heated fat, the effect of which he attributes to an oxidation product of cholesterol, but in over 1000 control rats up to 24 months of age kept in the same animal house he failed to observe any such tumours. Although he does not discuss *Cysticercus fasciolaris* as a possible etiological factor in his experiments, it would seem to be excluded by the absence of tumours in so large a number of controls. In three of our four cases of multiple mesenteric tumours in uninfested rats no liver tumour was present; in the fourth a small liver tumour was present, but it was not possible to determine which of the tumours was primary. Tumours associated with cysticerci occurred in three out of eleven infested rats in this experiment. As only one case of multiple mesenteric sarcomata of spontaneous origin was observed by us in a series of 300 *post mortem* examinations of uninfested rats in other experiments, the occurrence of these multiple tumours in animals fed with heated fats seems to be significant, despite the relatively small numbers of animals so far employed. The possibility that the multiple mesenteric tumours in Roffo's experiments and in ours were due to a chemical carcinogen therefore merits further consideration. Spindle cell tumours have been induced in rats, but not in mice, by subcutaneous injection of lard, heated to 350° C., or unheated (Burrows, Higer and Kennaway, 1936); spindle cell sarcomata were induced in mice by subcutaneous injections of cotton seed oil heated to 350° C. (Beck, 1941), and of sesame oil heated to the same temperature (Steiner, Steele and Koch, 1943). As most of the known chemical carcinogens are fat-soluble, it is to be supposed that they would follow the usual route of absorption for fat-soluble substances. In order to study the behaviour of a known carcinogen added to the diet, we fed rats with heated fat containing 2 per cent. 3 : 4 benzpyrene, and observed their organs at various times thereafter by direct ultra violet fluoroscopy. Animals killed from 1–4 hours after the ingestion of benzpyrene in this way showed fluorescence of the oesophagus, stomach and variable lengths of small intestine, and of the lacteals and cysterna chyli. It was possible to aspirate the fluorescent chyle, and to demonstrate in it spectrographically the presence of unchanged benzpyrene. During the same period the common bile duct showed a paler blue fluorescence, proving that the mechanism of excretion originally described after intravenous injection of benzpyrene (Picaud, 1936) was taking place. The submucous connective tissue and the lacteal and vascular capillaries appear to be the first mesoblastic tissue to be exposed to the

action of a fat soluble carcinogen administered in the diet. It is possible that the prolonged exposure of these mesoblastic tissues to a carcinogen may induce multiple primary tumours in somewhat the same way that multiple papillomata of the skin can be induced by repeatedly painting it with a carcinogen. The fact that three of our four tumours were spindle-cell sarcomata suggests that connective tissue of fibroblastic origin may be more susceptible to the action of a potential carcinogen in heated fat than is the endothelium. Carcinogenic action at a small distance from the point of maximum concentration of an injected carcinogen has frequently been observed by us (Peacock, 1933, and Peacock and Beck, 1938), and it seems possible that a fat soluble carcinogen could permeate the thin walls of the lacteals and so come in contact with surrounding areolar tissue in the mesentery. It should be noted, however, that in direct fluoroscopic observation of animals fed with benzpyrene the most striking fluorescence was seen in the intra abdominal adipose tissue, corresponding with a vascular distribution, and that direct leakage from the lacteals was not observed. The primary site of induction of spindle cell sarcomata may therefore be in the submucous connective tissue of the small intestine. Some examples of perforation of the intestine by invasion with spindle cell sarcoma might be taken to support this view. Further work will be necessary before the carcinogenicity of fats heated to about 350° C. can be definitely assessed, but the evidence points to the presence in them of some factor capable of inducing malignant mesoblastic tumours along the path of their absorption in addition to the benign gastric lesions already described.

SUMMARY.

1. Rats maintained on (*a*) adequate diet, (*b*) restricted diet (bread or potatoes and milk), to which were added lard heated to 220° C. or 350° C., developed multiple mesenteric sarcomata in 4 out of 71 animals surviving more than 300 days. These animals were not infested with *Cysticercus fasciolaris*, which is known to be associated with sarcoma in the liver of infested rats.

2. Some rats on the restricted diet also developed benign gastro papillomatosis.

3. Among 11 rats infested with *Cysticercus fasciolaris* 3 developed spindle-cell sarcomata attributable to the parasite.

4. Reasons are given for thinking that multiple mesenteric sarcomata in uninfested rats fed with heated fat were induced by a potential carcinogen acting on the mesoblastic tissues encountered in the process of absorption.

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Determination of Digestibility and Metabolizable Energy in Pure Fats and Discussion of Analytical Methods Employed in Experiments with growing Chickens

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During recent years the question regarding the usability of fats as addition to feed mixtures has been of particular interest. There are three reasons for this: (1) The high energy content of the fats makes it possible to produce concentrated feed mixtures. (2) Fats which contain essential fatty acids, have a growth promotive effect. (3) In the production of certain animal products it is possible to affect the composition of fatty acids in the product.

As far as poultry is concerned, addition of fat to the feed is used for all three reasons mentioned. For a complete utilization of the growth intensity of the broilers, it is necessary that the feed has a high energy content, on account of the high energy metabolism of the animals, compared to their weight. A supplement of unsaturated fat may come into consideration, when feed mixtures are used where the content of essential fatty acids is too low. Bælum & Petersen (1966) find a growth increase by addition of soya bean oil to a barley feed mixture, whereas no growth increase is found by addition of soya bean oil to a maize feed mixture. This is due to the fact that maize has a higher fat content than barley. Since poultry products have a high content of poly-unsaturated fatty acids, they are well suited in the human nutrition for covering the requirement for essential fatty acids. It has been demonstrated that the content of poly-unsaturated fatty acids in poultry products is easily increased by an addition to the feed of fat which contains the fatty acids in question. Vestergaard Thomsen (1965) finds that egg fat is enriched with poly-unsaturated fatty acids by feeding with soya bean oil and linseed oil.

During the last year a series of experiments with growing chickens has been conducted at this Department, partly in order to determine the content of metabolizable energy and the digestibility of pure fat, and partly to test the experimental procedure, with a view to forthcoming respiration experiments with growing chickens. In the experiment which will be discussed in the present paper, metabolizable energy as well as digestibility of lard, beef tallow, soya bean oil and linseed oil were determined. The tallow was obtained as cut tallow, it was thereafter ground and clarified, so that the product used was pure. The other three fats were obtained as pure fats.

Experimental plan

50-day-old chickens (White Plymouth Rock, cocks) were obtained. For running the experiments 16 of the 50 were taken at random, after about 1 week; they were placed individually in the experimental cages, and thus they were adjusted before the beginning of the experiment. The experiment started, when the chickens were 19-days old. Prior to the beginning of the pre-experimental period, the 16 chickens were distributed in four groups, each group consisting of four chickens, and it was attempted to make the distribution in such a way that the same average weight and a uniform standard deviation of the

[1] Norges Landbrukshøgskole, [illegible]

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Table 1. *Weight and distribution in groups of the chickens at the beginning of the experiment*

Group / Treatment	1 Soya bean oil	2 Tallow	3 Lard	4 Linseed oil
Weight, g	287	281	255	309
	265	259	276	264
	262	251	292	276
	282	290	256	277
Average weight	274	270	270	282
Standard deviation	12	18	18	19

weights for the four groups were obtained. The weights of the four groups at the beginning of the experiment are given in Table 1.

Feeding plan

The experiment was carried through in five periods, and during the first and the fifth period a basal mixture was given. In the second, third and fourth period the basal mixture with added 6, 12 and 18% fat, respectively, was given. Thus a basal feed mixture and twelve feed mixtures with fat were prepared. A gradual feeding was used, the feed quantity being increased from day to day. This experimental method is described by Thorbek (1965). As the feed strength was further increased from period to period, the principles of gradual feeding was not followed. The feeding was [illegible] than [illegible] a pre-[illegible] could consume the full ration given in each feeding. Feed was given twice daily, at 8 a.m. and 4 p.m., and water was available *ad libitum*.

The feeding plan is represented graphically in Figure 1.

Composition of the feed

Large quantities of fat were added to the feed, which thereby became very rich in energy. For this reason, the basal feed mixture had to be enriched with protein. As protein sources were used soya bean meal and herring meal, to make sure that the protein had a high biological value. The composition of the basal feed is given in Table 2.

By feeding with large quantities of fat a vitamin-E deficiency may occur, and an extra supply of vitamin E was added. Similarly, the addition of antioxidant was meant to prevent oxidation during storage.

Collection of faeces

Every morning before the feeding the faeces was collected quantitatively in plastic boxes with covers, and stored in a freezer immediately after the collection. Nevertheless, a faint gas production could be observed in some of the samples; the loss must, however, be considered as negligible.

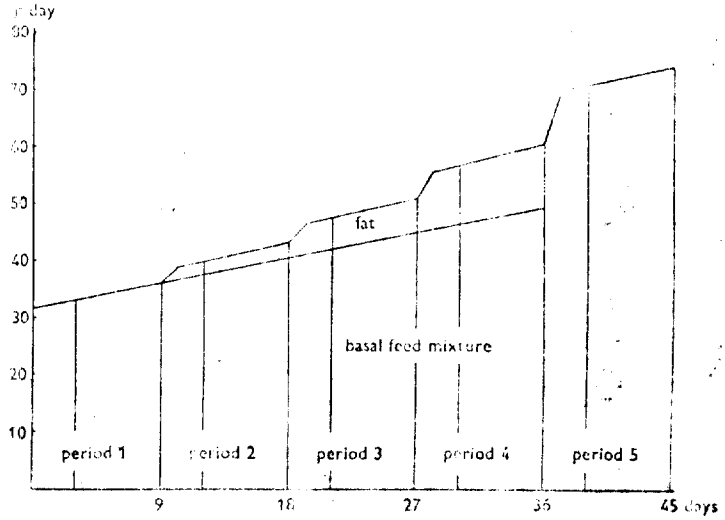

Fig. 1. Feeding plan.

[illegible]

Just before taking out samples [illegible] the [illegible] [illegible] [illegible] [illegible] for determination of dry matter, [illegible] and faeces-N. Samples of about 120 g of the faeces were freeze-dried and ground on a plate mill for determination of fat and energy. The first four analyses mentioned were made in accordance with the analytical precepts of the laboratory (Weidner & Jakobsen, 1962). As this experiment primarily serves to determine metabolizable energy in fat and the digestibility of fat, the energy determinations and the fat analyses will be mentioned more detailed and discussed on the basis of the results of the experiments.

Energy determination

The determination of the energy in feed and faeces was made on an adiabatic calorimeter (Gallenkamp). The principal difference between an adiabatic calorimeter and the older calorimeters of the Department is that the water-jacket surrounding the calorimeter bucket, by means of thermostatic control is heated with the rise of temperature in the calorimeter bucket, so that no heat exchange with the environment takes place. Similarly, the reading work is facilitated, as the temperature is only read twice, as compared with 8–10 times on the older calorimeters. In the determinations of energy in the pure fat the fat was weighed in the crucible, and on the ignition wire a small cotton thread (0.004–0.005 g) was placed, which served as pre-ignition of the fat. Without this arrangement it was very difficult to ignite the pure fat samples.

Table 2. *Composition of the basal feed (in per cent)*

[illegible]	7.00 %
Maize	50.00
Soya bean meal	32.00
Herring meal	8.00
Vitamin mixture[1]	0.50
Dicalcium phosphate	2.50

Crude protein: 24.4%. Crude fat: 3.0%
Gross energy: 4.481 kcal/g dry matter

[1] Composition of the vitamin mixture: 1 g contains: vitamin A, 1600 I.U.; vitamin D_3, 400 I.U.; vitamin B_1 260 µg; vitamin B_2 640 µg; nicotinamide, 3700 µg; D-pantothenic acid, 340 µg; α-tocopherol-acetate, 1800 µg; 1,2-dihydro-6-ethoxytrimethylquinoline, 20 mg.

Analyses were made on all feed mixtures. It appeared that the results from the analysis were somewhat lower than the calculations made on the basis of basal feed and the pure fat, respectively. The reason for this may be ascribed to the fact that the pelleting of the very fatty samples prior to burning, may squeeze out fat. Therefore the energy content of the feed was calculated on the basis of energy determinations in the basal feed and the pure fat separately. The results of the analyses of the feed mixtures and the values calculated on the basis of analyses on basal feed mixture and fat, separately, are given in Table 3.

Energy content of the fat

Soya bean oil:	9.430 kcal/g
Tallow:	9.484 kcal/g
Lard:	9.424 kcal/g
Linseed oil:	9.430 kcal/g

Table 3. *Energy content of the feed mixtures (in kcal)*

	Soya bean oil		Tallow		Lard		Linseed oil	
	Total mixture analysed	Basal mixture and fat analysed	Total mixture analysed	Basal mixture and fat analysed	Total mixture analysed	Basal mixture and fat analysed	Total mixture analysed	Basal mixture and fat analysed
	kcal							
Basal mixture + 6% fat	4.693	4.813	4.726	4.820	4.780	4.820	4.726	4.[illegible]2
Basal mixture + 12% fat	5.028	5.138	5.018	5.148	5.045	5.149	5.005	5.147
Basal mixture + 18% fat	5.297	5.479	5.4[illegible]	5.47[illegible]	5.3[illegible]2	5.47[illegible]	5.[illegible]8	5.[illegible]7

Digestibility coefficients for crude fat

	Chicken no.	Basal mixture Period 1	Period 5	Basal mixture + 6% fat Period 2	Basal mixture + 12% fat Period 3	Basal mixture + 18% fat Period 4
[illegible] oil	1	78.0	75.8	90.8	95.8	96.5
	2	80.1	78.1	92.4	95.4	95.7
	3	79.5	77.5	91.8	94.0	95.2
	4	80.2	76.3	92.0	95.9	96.2
[illegible]	5	79.6	79.6	81.8	[illegible]	91.7
	6	75.4	77.1	86.1	83.8	87.7
	7	76.3	77.4	86.9	88.0	91.6
	8	73.8	76.9	85.9	87.2	83.8
[illegible]	9	77.1	78.4	90.3	92.6	92.7
	10	76.4	77.3	88.9	93.3	89.5
	11	78.4	76.7	85.1	94.6	92.5
	12	74.3	74.3	88.4	93.5	90.2
[illegible] oil	13	79.2	76.7	89.3	94.2	96.1
	14	75.4	76.6	91.7	92.3	95.4
	15	79.9	76.8	93.3	95.4	96.0
	16	79.6	77.5	92.2	94.4	96.1
	$\bar{x}$	77.7	77.1	89.2	92.3	92.9
Standard deviation		2.2	1.2	1.8	1.3	2.1
Standard deviation p.c.		2.8	1.6	2.0	1.4	2.3

[a] Fat analysis lost.

The figures stated in Table 3 show the condition mentioned, and it seems further that the difference is greatest with liquid fats. As for the faeces, both energy determinations and fat analyses were carried out on the freeze-dried samples.

Fat analyses

The accuracy for determining digestibility of fat depends as well on the storage of the analytical samples and their preparation prior to the analysis as on the analytical method itself. Both during the storing of the samples and during the drying the fat fraction may undergo considerable changes. By a heat-drying of the wet material prior to the analysis an oxidation of the fat may take place. Such an oxidation will especially occur at the double linkages in the carbon chain of the unsaturated fatty acids, and as the extent of the oxidation will probably be different from sample to sample, the standard deviation of the analytical results is increased. Madsen (1963) and Geardbo Thomsen (1967) find a rather great standard deviation of the digestibility coefficients for crude fat, where the analyses are made on [illegible] samples. In the present experiment where the analyses are made on freeze-dried samples, the standard deviation of the digestibility is lower. It lies between 1.5% – 3% as well for the basal feed mixture with the low fat content as for the fatty mixtures. The digestibility coefficients for crude fat are given in Table 4.

From Table 4 it will be seen that the digestibility coefficients for the basal feed are from 77.1–77.7, and that the digestibility in the periods 2, 3 and 4 is considerably higher.

There is no reason to assume that the fat in the basal feed has a lower true digestibility than the fat added, but since some endogenous fat is excreted, and part of the bile pigments are dissolved by the extraction, the apparent digestibility will be lower when the fat quantities in the feed are small.

In period 1 the standard deviation of the digestibility coefficients is greater than in period 5, where the chickens were also given the basal feed. This condition may be due to the fact that the samples for period 1 were stored at room temperature after finishing freeze-drying until analysing took place, whereas all other samples were stored in deep freeze after freeze-drying

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Table 5. [illegible] and digested quantities of pure fat

	Chicken no.	Period 2	Period 3	Period 4
Consumed fat, g/day		2.51	5.97	10.59
		Digested fat, g/day		
Soya bean oil	1	2.44	5.58	10.43
	2	2.47	5.82	10.31
	3	2.45	5.72	10.26
	4	2.46	5.86	10.38
Tallow	5	2.08	--	9.92
	6	2.27	5.09	9.48
	7	2.29	5.39	9.95
	8	2.24	5.35	9.02
Lard	9	2.41	5.72	10.02
	10	2.37	5.78	9.65
	11	2.22	5.87	10.00
	12	2.38	5.83	9.77
Linseed oil	13	2.36	5.83	10.37
	14	2.47	5.72	10.31
	15	2.50	5.91	10.36
	16	2.46	5.83	10.37

in order to prevent a possible oxidation during the storing of the samples.

A reason for the change in the level of digestibility coefficients and a change in the standard deviation may be ascribed to the analytical method itself. It may here be a question of using different extraction methods, where the different fat fractions have a different solubility. Vestergaard Thomsen (1965) has investigated the difference in solubility of fat from chicken faeces by ether extraction and by chloroform extraction, and finds that more crude fat is dissolved by chloroform extraction, and finds consequently lower digestibility coefficients. Part of this difference may be due to the greater solubility of the bile pigments in chloroform.

The fat analyses are carried out in accordance with the precepts of the Department, with the modification that the samples are dried to constant weight under vacuum and nitrogen flow for 22 hours prior to the extraction. This analytical technique is described by Weidner (1967). Just Nielsen (1967) finds a difference in the digestibility of crude fat [illegible], where the fat analyses on the [illegible] made in accordance with the [illegible] of the Department and partly after drying for 22 hours under vacuum and nitrogen flow prior to the extraction. In the analyses made according to this last-mentioned method, the [illegible] from 67.5 to 72.7, [illegible] smaller quantity of crude fat was found in the faeces and like wise the standard deviation of the digestibility coefficients decreased. These analyses were made on heat dried samples. The conclusion of the above described conditions is that freeze-drying of the samples compared with heat-drying reduces the standard deviation of the digestibility coefficients and that the digestibility coefficients are depending on the analytical method used.

Another factor which affects the digestibility of the fat is the calcium content of the feed. Pettersson (1964) finds, in experiments with pigs that the digestibility of the fat decreases with an increasing calcium content in the feed, as a precipitation of the calcium salts of the fatty acids takes place in the digestive tract, and finds that such a saponification occurs too, when the feed has a normal content of calcium. This condition particularly applies to the long-chained saturated acids: myristic acid, palmitic acid and stearic acid, whereas the digestibility of oleic acid, linolic acid and linolenic acid is only affected to a smaller degree. By the usual fat analysis with ether extraction the precipitated calcium salts are withdrawn, due to their insolubility in ether, and the fat content in the faeces is determined too low. Hereby, a higher digestibility coefficient will be determined, despite of the lower digestibility of the fat. In the experiments carried out by Pettersson, the fat extraction by the analysis was made after a preceding hydrolysis with 7.5 *n* hydrochloric acid.

Digestibility of the pure fats

On the basis of the digested quantities of crude fat in the basal feed and the digested quantities in the total feed, the digested crude fat from the four different kinds of fats has been calculated for each chicken separately in the three periods. The consumed and the digested quantities of the four kinds of fats are given in Table 5.

On the basis of the figures given in Table 5 the digestibility coefficients are calculated for the individual fats according to the regression method, and are given in Table 6.

Table 6. *Digestibility coefficients for the pure fats*

	Digestibility coefficient	[illegible]	[illegible]
Soya bean oil	97.7	0.5	[illegible]
Beef tallow	91.3	2.4	[illegible]
Lard	92.7	1.5	[illegible]
Linseed oil	97.8	[illegible]	[illegible]

7. *Metabolizable energy per g consumed dry matter (in Kcal)*

	Chicken no.	Period 1 Basal mixture	Period 2 Basal mixture 6% fat	Period 3 Basal mixture 12% fat	Period 4 Basal mixture 18% fat	Period 5 Basal mixture
		Kcal				
Soya bean oil	1	3.348	3.769	4.142	4.552	3.324
	2	3.267	3.747	4.150	4.525	3.358
	3	3.289	3.715	4.099	4.501	3.298
	4	3.343	3.766	4.159	4.570	3.330
Tallow	5	3.287	3.692	3.901	4.182	3.311
	6	3.303	3.606	3.901	4.092	3.362
	7	3.338	3.713	3.907	4.165	3.347
	8	3.302	3.650	3.870	4.015	3.369
Lard	9	3.304	3.755	4.031	4.370	3.337
	10	3.288	3.720	4.045	4.237	3.337
	11	3.328	3.731	4.072	4.357	3.317
	12	3.289	3.743	4.036	4.322	3.337
Linseed oil	13	3.359	3.694	4.171	4.545	3.355
	14	3.271	3.646	4.103	4.472	3.392
	15	3.302	3.750	4.194	4.507	3.313
	16	3.273	3.554	4.147	4.526	3.317
$\bar{x}$		3.306	3.703	4.058	4.371	3.338
Standard deviation		0.029	0.050	0.027	0.053	0.025
Standard deviation p.c.		0.9	1.4	0.7	1.2	0.7

It will be seen that the digestibility coefficients are highest for the two liquid fats, soya bean oil and linseed oil. These two have the highest content of unsaturated fatty acids. The other two fat sources, tallow and lard, have a lower digestibility due to a high content of palmitic and stearic acid.

The standard deviation is highest for tallow and lard, which was to be expected, due to the difficulties in obtaining a completely homogeneous mixture of the basal feed mixture added to these two fats. This was specially the case with tallow.

The content of metabolizable energy

In the first and the fifth period of the experiment the metabolizable energy in the basal feed was determined. On the basis of the metabolizable energy in the basal feed and in the total feed in [illegible] 2, 3 and 4, the metabolizable energy [illegible] can be calculated. The metabolizable energy was, by means of determination of nitrogen deposition, corrected to a nitrogen [illegible] of the nitrogen [illegible] [illegible] differences, it was sufficient to correct to a fixed nitrogen deposition. For a deposition of 45%, only small corrections were necessary. The metabolizable energy per g dry matter, corrected to 45% nitrogen deposition, is stated in Table 7.

For the periods 2, 3 and 4, [illegible] the inner standard deviation.

From the low standard deviations of the metabolizable energy it will be seen that the energy determinations are rather safe analyses. In digestibility experiments with pigs, Madsen (1963) finds, by the determination of the digestibility of energy, a low standard deviation. Similar results are found by Gaardbo Thomsen (1967) in digestibility experiments with hens.

On the basis of the results obtained from the five periods, the metabolizable energy of the pure fats has been determined. Metabolizable energy in the basal feed had been calculated as average of the periods 1 and 5. The values for the pure fats in the periods 2, 3 and 4 thus occur as differences between the total metabolizable energy and the metabolizable energy due to the basal feed. The metabolizable energy of the pure fats is given in Table 8.

[illegible] of the [illegible] given in Table [illegible]

Table 8. *Metabolizable energy in [illegible] pure fat (in kcal)*

	Chicken no.	Period 2	Period 3	Period 4
Consumed fat, g/day ...		2.51	5.97	10.59
		Metabolizable energy, kcal/day		
Soya bean oil	1	24.22	55.74	97.75
	2	24.43	57.15	92.54
	3	23.73	[illegible]	96.91
	4	24.17	[illegible]	98.67
Tallow	5	22.50	46.30	82.62
	6	19.19	43.99	76.42
	7	21.81	43.89	79.86
	8	19.70	43.49	72.17
Lard	9	24.04	51.04	90.01
	10	23.04	51.94	83.39
	11	23.08	52.75	8[illegible].24
	12	23.82	51.52	8[illegible].80
Linseed oil	13	22.74	58.95	[illegible].59
	14	21.79	53.88	[illegible].42
	15	26.46	58.81	[illegible].30
	16	23.14	52.23	[illegible].85

the metabolizable energy per g fat is calculated by means of a regression analysis. The results are given in Table 9.

The results show that the metabolizable energy for soya bean oil and linseed oil is very high, which corresponds with the high digestibility coefficients for these fats. The metabolizable energy is lower for lard, and lower for tallow.

In Table 10 the direct determined digestibility coefficients are compared with digestibility coefficients calculated after the metabolizable energy.

By a comparison between the digestibility coefficients and the metabolizable energy it appears that, as far as lard and tallow are concerned, the digestibility coefficients are higher, whereas there is agreement, as regards linseed oil and soya bean oil. This condition may be due to the fact that, as far as lard and tallow are concerned, a precipitation of the calcium salts of the fatty acids (palmitic and stearic acid) has taken place, and that the fat content of the faeces has thus been determined too low by the analytical method used.

Table 9. *Metabolizable energy per g pure fat*

	kcal/g	s_x	s
Soya bean oil	9.10	0.06	0.67
Beef tallow	7.05	0.24	2.20
Lard	7.92	0.15	1.78
Linseed oil	9.07	0.18	2.01

Table 10. *Digestibility coefficients directly determined versus digestibility coefficients calculated after the metabolizable energy*

	Soya bean oil	Beef tallow	Lard	Linseed oil
Determined	97.7	91.3	92.7	97.8
Calculated	96.5	74.3	84.0	96.1

Summary

Experiments have been carried out for determination of the metabolizable energy as well as for the digestibility of four pure fats: Soya bean oil, beef tallow, lard and linseed oil. The analyses of the faeces are made on freeze-dried samples. By a comparison with experiments conducted by other authors, freeze-drying versus heat drying of samples of faeces causes as well a change in the digestibility coefficients as a small standard deviation. The metabolizable energy is highest, as far as the two liquid fats are concerned. As for these two fats, there is agreement between the metabolizable energy and the digestibility, whereas the digestibility coefficients for tallow and lard are found to be too high in relation to the metabolizable energy. This condition may be due to a precipitation of calcium soaps of the saturated, long-chain fatty acids when these salts are withdrawn from the determination by the analytical method employed.

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Long-Term Responses of Rats to Heat-Treated Dietary Fats: IV. Weight Gains, Food and Energy Efficiencies, Longevity and Histopathology

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ABSTRACT

Representative cottonseed salad oils, corn oils, lards and hydrogenated vegetable shortenings, and portions of the same fats heated at 182 C for 120 hr were fed as 20% of nutritionally adequate diets to weanling albino rats in longevity studies. Differences in the responses of rats fed diets containing the unheated and heated fats were generally small with respect to rates of gain, 12th week and adult weights, efficiencies of utilization of absorbed energy, incidences of grossly detectable diseases and longevities. There were no indications that the feeding of the heated fats had shortened survival times in comparison with the comparable unheated fat. Animals fed hydrogenated vegetable shortening, heated or unheated, survived the longest. However, gains were slightly slower with the heated cottonseed oil diets, and food efficiencies were slightly lower with the heated cottonseed oil and heated lard diets because of decreased digestibilities of these fats. The usual disabilities of old age such as nephritis, respiratory disease and periarteritis were present in all groups. The incidence of mammary tumors was high but did not differ significantly with the kind of fat, heated or unheated. Tumor incidence other than mammary was similar in both sexes and there was no significant difference between fresh and heated fats. Absence of adverse effects attributable to the heated fats during the life span of the rats is further evidence of the safety of these fats of the quality customarily consumed by the human population.

INTRODUCTION

Edible fats undergo chemical and physical changes when exposed to heat/or oxygen or both and, if the treatment is severe enough, some of the products may be harmful to experimental animals when fed under appropriate conditions. The nature and extent of the changes have been investigated extensively during the last few years, yet because of the complexity of the mixtures, there is still much to be learned regarding the composition and the biological effects of heated or oxidized fats. Three general types of chemical changes occur: oxidation, cleavage to smaller compounds and polymerization.

There is considerable evidence that the treatments to which edible fats may be subjected during practical home or institutional use do not cause the formation of deleterious substances in amounts sufficient to impair health (1-15). On the other hand, several teams of scientists have observed adverse biological effects when fats which had been severaly heated (2,11-13,16-25) or extensively oxidized (12,23-42) were fed or injected. Toxic substances can be concentrated in certain fractions of the treated fats by distillation or urea adduction procedures (12,16-21,28,30-37). Fats containing relatively large proportions of unsaturated fatty acids, particularly polyenoic acids, yield more of these toxic substances than the saturated fats (20,21,30). Factors in addition to unsaturation appear to be involved since in our experience different lots of a single type of oil yield reproduceably differing amounts of urea non-adducting fraction (NAF). In general, the biological effects of heated or oxidized fats are in proportion to the NAF content (25,27,31), but the effects differ depending on the nature of the fraction. Concentrates that are predominately monomeric tend to be absorbed more readily and are more toxic than dimers or longer chain polymers (12,20,31).

The small amounts of lipid-derived products developed in fats during cooking ordinarily cannot be detected biologically without extraction and concentration of the NAF. Furthermore, substantial amounts of severely heated fat can be included in the diet without adversely affecting growth or well-being of rats, particularly if associated with some unchanged fat (5,16,35,36).

Nevertheless, the presence in foods of even small amounts of possible deleterious substances, such as NAF, is cause for concern until there is sufficient evidence that these amounts are harmless. As mentioned above, existing data support the belief that fats used in foods or in cooking of foods are safe in this respect, even

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though other evidence makes it quite clear that abuse of fats by excessive exposure to heat or oxygen will result in formation of toxic products. For the most part previous biological tests have been of short duration and may not have detected substances that would elicit a response on prolonged administration or in massive doses. It was to study the cumulative and chronic effects of heated fats, if such effects exist, that the present experimental work was designed and conducted. The findings, as reported, confirm the conclusions of the short-term tests, i.e., heat treatments more severe than those used in cooking foods yet in a practical range do not result in formation of sufficient quantities of fat-derived products to cause serious adverse biological effects. They are in substantial agreement with the observations of Nolen et al. (15) who conducted longevity studies with rats fed fats heated in the presence of foods.

EXPERIMENTAL PROCEDURES

In order to obtain the information desired, uniform groups of rats were fed diets which contained either a typical commercial fat or portions of this fat which had been subjected to a relatively severe heat treatment. Responses of the animals were compared with respect to: (a) rate of growth, (b) ability to digest the fats, (c) efficiency of food and energy utilization, (d) incidence of various abnormalities during life, (e) longevity, (f) gross changes observed at autopsy, and (g) organ weights. Tissues were taken at autopsy and preserved for subsequent histopathological study in the Human Nutrition Research Division, ARS, USDA.

Four widely used dietary fats were selected: cottonseed salad oil (CS), corn oil (CO), lard (L) and hydrogenated vegetable oil shortening (S). Three prominent brands of each were purchased during the course of the study in the necessary quantities in regular packages (38-50 lb) from commercial suppliers. Equal aliquots of the three brands of a specific fat were blended together for use in the diets and for heating prior to use. Both heated and unheated samples were stored in filled, sealed glass jars at 3 C until used.

Heat Treatment

Fats were heated at 182 C continuously for 120 hr in 46 lb quantities in a commercial, electrically heated deep fat fryer equipped with a chromed steel container and a stainless steel clad heating element. When used in this quantity there were 6.5 sq in. of surface exposed to air for each pound of fat heated, a ratio typical for this type of equipment. During heating the fats were stirred gently to avoid local differences in temperature. The stirring was not vigorous enough to cause entrapment of air bubbles, but it did provide a continuous change of the surface exposed to air and to the heating element. Fats treated in this manner are designated as HCS, HCO, HL and HS to differentiate them from the unheated CS, CO, L and S.

This relatively long and severe heat treatment was considered to be a compromise between short-term, single use of fats which would result in only minimal changes that might not be detectable biologically and more drastic treatments that have no practical counterpart. It was known from previous work (12,25,31) that such a treatment would be severe enough to decrease the digestibility of CS, the fat which had been most extensively studied. On the other hand, rats fed diets containing fats heated under similar conditions were known to grow well and to be apparently healthy for periods up to 12 weeks (11). It was also known that CS treated in this manner would be beyond practical commercial usage, as it would foam violently if attempts were made to introduce foods such as potatoes (12,14,42,43).

Certain analytical characteristics of the fats are shown in Figure 1. The general increases in viscosity and in the content of the urea non-adducting fraction, and the decreases in linoleic acid content and iodine values are greater than is typical for such fats used in practical food frying operations. Peroxide values were low and changed little by the heating procedure.

Preparation and Composition of Diets

Nutritionally complete, synthetic-type diets including 20% of the various test fats were prepared by adding appropriate quantities of the fats and 1% of wheat germ oil to aliquots of a uniform supply of a basal diet mix having the following composition: purified casein, 20.1; purified lactalbumin, 10.0; Jones and Foster salt mixture, 4.0; cellulose (Cellu Flour), 2.0; sucrose, 35.2; primary grown yeast, 5.0; d-α-tocopherol dry mix (Myvamix, 44 I.U./g), 1.0; choline chloride dry mix (25%), 0.4; vitamin A dry mix (Stabmix A, 10,000 I.U./g), 0.2; vitamin D_2 dry mix (Daves Sterol D-2, 1500 I.U./g) 0.2; and vitamin premix, 1.0. The vitamin premix contained, in mg/g, riboflavin, 0.45; niacin, 1.5; d calcium pantothenate, 3.5; pryidoxine hydrochloride, 0.65; biotin, 0.033; 2 methyl naphthoquinone, 1.0; *p*-aminobenzoic acid, 10.0; vitamin B_{12}, 0.001; and sucrose sufficient to bring the total to 1.000 g. The first five com-

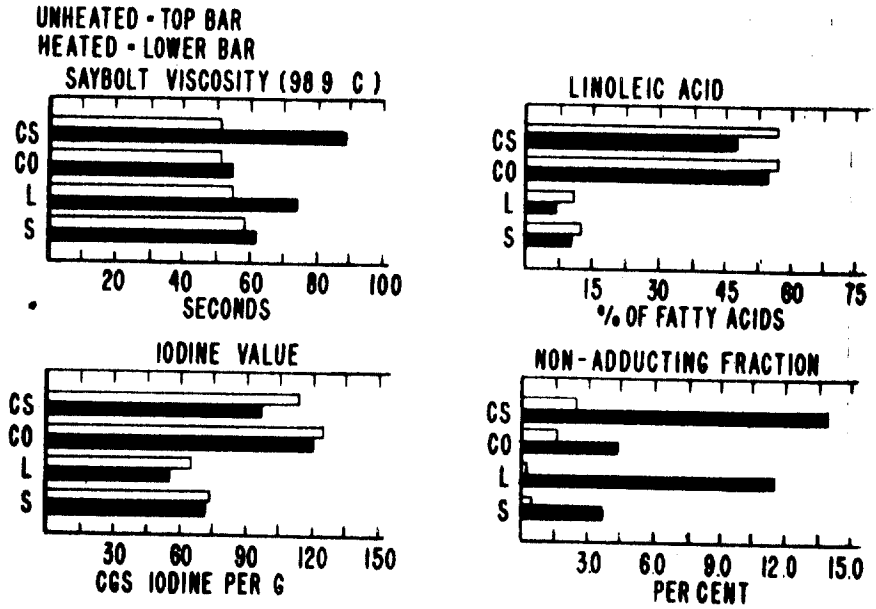

FIG. 1. Analytical values of fats before and after heating.

ponents of the basal diet were premixed in advance (premix 1 stored at room temperature), and the remaining six were likewise premixed (premix 2 stored in brown bottles at 3 C).

The diets were made by first mixing the proper aliquots of premix 1 and premix 2, then adding, during mixing, the appropriate melted fat and, finally, the wheat germ oil. Analyses of the diets gave average values as follows (in per cent): moisture, 3.3; protein, 27.2; fat, 21.9 and ash, 3.6. Fresh batches of each diet were prepared at intervals of about two weeks. Weighed quantities of fresh food in 3 or 6 oz widemouth glass jars were supplied three times weekly to the animals during the growth period and at least weekly thereafter. Food and water were supplied ad libitum. Uneaten food was weighed to determine food usage.

Care of Animals

Fifteen replications, each comprising one male and one female weanling rat (Holtzman albinos) for each unheated fat diet group and two of each sex for each heated fat diet group, were started weekly on the test diets for 15 weeks. Rats were caged individually in drawer-type cages with wire mesh fronts and bottoms. The rats were randomly distributed in cages suspended on racks in an air conditioned room with temperature controlled at 25-26 C and humidity at 45-50%.

Animals were weighed at about the same time each Wednesday. At that time uneaten food was removed for weighing, and each animal was inspected carefully for disease or physical abnormalities. A few rats with rales or suspected middle ear infections were isolated, but no treatment of any kind was administered to the animals except for trimming a few elongated incisors when necessary. Animals which showed severe or consistent losses of weight or other conditions that indicated ill health were observed more frequently. Those which became obviously moribund were decapitated and autopsied immediately to assure tissues suitable for histopathological evaluation. Animals which were found dead were autopsied as soon after death as possible.

During the 2nd, 12th and 52nd week of feeding, complete collections of feces were made for 10 randomly selected rats in each of the dietary groups. Lipid was extracted with ethyl ether from the pooled, acid hydrolyzed sample for each group for each period and weighed following solvent removal. Fat intakes for the same rats were calculated from the food intake and food composition records. The difference between the total dietary fat intake and the total fecal fat output represents gross fat absorption (uncorrected for endogenous fat). The weights of fat absorbed divided by weights of fat consumed gives practical digestibility values.

When the animals were 22 weeks of age, two males and two females which had been pre-

TABLE I

Average Weights of Rats Fed Diets Containing Unheated or Heated Fats for 12, 52 or 78 Weeks

Fat in diet	Average weights of rats in grams					
	Males			Females		
	12 wk	52 wk	78 wk	12 wk	52 wk	78 wk
CS[a]	451	655	681	268	385	458
HCS[b]	427[c]	634	674	253[c]	380	455
(CS/HCS) x 100	106	104	101	106	101	101
CO[a]	432	666	697	260	391	469
HCO[b]	431	653	723	265	394	464
(CO/HCO) x 100	100	102	96	98	99	101
L[a]	436	613	632	257	367	428
HL[b]	430	638	722	256	365	440
(L/HL) x 100	102	96	88	100	101	97
S[a]	440	643	680	262	358	442
HS[b]	428	615	648	254	361	425
(S/HS) x 100	103	105	105	103	99	104
All unheated	440	644	672	262	375	449
All heated	429	635	692	257	375	446
(Unheated/heated) x 100	103	101	97	102	100	101

[a]Fifteen animals started in each group.

[b]Thirty animals started in each group.

[c]Significantly less ($P < 0.05$) than for rats fed the unheated oil. Data for the 78 week periods were not statistically analyzed because of the small and irregular group sizes.

selected randomly for each group were killed for gross and histopathological examination. This procedure was repeated with other pairs of animals at ages of 36 and 50 weeks.

Except for the six randomly selected animals in each group killed for autopsy and tissue samples, as just described, all animals were maintained on their respective diets until they died or became moribund, or until a preselected termination point based on reduction of the colony to about 10% of the starting number was reached. At this time (928 days of age), all survivors were killed for termination gross and histopathological observations. At the time of autopsy, observations regarding the gross appearance of the animal and its organs and tissues were recorded; designated organs (liver, kidneys, adrenals) were removed, weighed and preserved in 10% neutral formalin for possible future histopathological study. All autopsies for this experiment were coded, i.e., done without knowing the diet group to which the animal belonged.

RESULTS AND DISCUSSION

Two general types of observations were made with respect to the influence of heating on the wholesomeness of fats, those relating to the effectiveness and efficiencies of the several fats tested as measured by growth and food utilization, and those relating to the health and longevity of the animals. Neither of these criteria gave any indication that fats heated for 120 hr at 182 C adversely affected the general health or well-being of rats to which they had been fed for their full life span. There were some minor differences between groups when heated-unheated comparisons were made, but these tended to balance out, some being in favor of the heated, others in favor of the unheated.

Growth

Animals fed heated fats grew well and at about the same rates as their counterparts fed unheated fats and maintained their weights over the long-term, as shown by the data in Table I. The only effect noted was a slightly slower rate of gain for each sex in the HCS diet group ($p \leq 0.05$ at 12 weeks, not significant thereafter). The slightly slower rate of growth for HCS fed rats is consistent with previous observations (21,25,31). There was generally more variation

TABLE II

Food and Calorie Efficiencies of Rats Fed Unheated or Heated Fats for 12 Weeks and Digestibility of Fats Fed

1	2	3	4	5	6	7	8	9
Sex and diet type	Average 12 wk gain, g	Average total 12 week food disappearance, g[a]	Grams food consumed per gram gain	Calories absorbed per gram gain[b]	Digestibilities[c] of fats in the diet, %			Digestible energy per gram diet, calories[d]
					2nd wk	12th wk	52nd wk	
Males								
CS	398	1219	3.06	14.4	91.6	92.3	93.7	4.70
HCS	376	1231	3.28	14.3	80.0	76.3	65.9	4.37
CO	381	1175	3.08	14.6	93.6	92.8	93.3	4.71
HCO	380	1192	3.14	14.6	91.1	90.2	89.8	4.66
L	385	1186	3.08	14.6	91.0	92.0	93.7	4.73
HL	378	1265	3.34	15.0	76.1	79.9	77.4	4.48
S	385	1244	3.23	14.6	86.0	83.7	89.3	4.52
HS	376	1216	3.23	14.6	86.7	84.1	76.8	4.53
All unheated	387	1206	3.11	14.5	—	—	—	4.66
All heated	378	1226	3.25	14.6	—	—	—	4.51
Females								
CS	217	874	4.02	19.1	93.7	94.2	95.0	4.74
HCS	203	922	4.55	20.0	79.7	78.1	72.6	4.41
CO	210	869	4.14	19.6	95.4	94.8	94.2	4.75
HCO	214	900	4.21	19.7	92.2	91.9	91.7	4.69
L	206	864	4.19	20.0	92.6	94.4	94.6	4.78
HL	206	915	4.44	20.1	75.9	82.3	79.1	4.53
S	211	906	4.30	20.0	88.6	91.0	79.0	4.67
HS	204	883	4.33	20.0	86.4	88.6	82.2	4.62
All unheated	211	878	4.16	19.7	—	—	—	4.73
All heated	207	905	4.38	19.9	—	—	—	4.56

[a]Food disappearance corresponds closely with food consumption, as only a few crumbs were scattered and not weighed.

[b]Calculations based on digestible energies shown in the last column in this table.

[c]See text.

[d]Calories were calculated using average diet composition and factors of 3.95 for sucrose, 4.27 for protein, 9.30 for vegetable oil and 9.50 for lard, according to USDA Handbook 8, (44). The 9.30 and 9.50 combustible energy values were used for fat, because digestibility was based on data for 12 week fat digestibilities listed in Column 7.

between the types of unheated fats fed than there was between the heated vs. unheated variants of each type. The long-term weight differences between the four types of unheated fats fed were never more than 9% and were not significant statistically at any age.

Food Efficiency and Digestibility

At the end of 12 weeks on diet the rats fed HCS or HL had consumed more food per gram gain than animals on diets containing unheated fats of the same types, but those fed HCO or HS had not consumed more than their counterparts (Table II, Column 4). The increased food consumptions were statistically significant ($P \leq 0.01$) for the males and females fed HL and for the females fed HCS. However, when the comparisons were made on a per calorie absorbed basis rather than on a per gram of food consumed basis, the differences between the heated and unheated fats disappear (Table II, Column 5). It seems probable that the decreased food efficiencies of animals fed diets containing HCS or HL are due to nondigestible substances in the fats, presumably polymers originating during the heating of the fats (19,20,31). The presence of nondigestible lipids is demonstrated by the digestibility values for each of the three time periods (Table II, Columns 6, 7 and 8). Substantial decreases in digestibility for HCS vs. CS and HL vs. L were observed for all periods. The digestibility values are similar for the 2nd, 12th and 52nd week for each unheated fat, however, there is some indication that the heated fats were progressively less well digested as the animals aged. The statistical significance of the differences observed could not be determined because it was necessary to pool feces for rats in each group to provide sufficient sample for analysis.

The equal utilizations of energy other than that of the nondigestible portions of the fats is evidence that digestibilities of the other components of the diets were not affected. These observations differ from those of Friedman et al. (31) who, using cottonseed oil stirred for 190 hr at 225°C, found that "This difference in nutritive value (i.e., energy) cannot be accounted for by the decreased digestibility of the heated cottonseed oil." However, their fat, having been much more severely treated, was not comparable to those used in our tests.

Incidence of Diseases or Abnormalities

In general the animals remained healthy and lived long, relatively disease-free lives but, as expected with a group of aging animals, a variety of physical abnormalities was noted. These observations were recorded in detail and tabulated according to type and treatment of the fats and sex of the animal. The abnormalities were typical of those occurring in the rat and there were no substantial differences in frequencies between types of fat or treatments.

TABLE III

Survival of Rats Fed Diets Containing Unheated or Heated Fats

Dietary fats	Average survival in rat days		
	M	F	M & F
CS	635	738	687
HCS	650	706	678
Both	646	715	680
CO	656	683	669
HCO	657	706	681
Both	656	700	678
L	551	808	679
HL	662	696	679
Both	632	727	679
S	667	767	717
HS	691	765	728
Both	684	766	725
All unheated	627	749	688
All heated	665	718	692
Entire experimental colony	646	734	691

Longevity

The overall survival of the rats in this experiment was good, an average of 646 days for the males and 734 days for the females, and there were no indications that animals fed heated fats had shortened lifespans. In fact, in five of the eight heated-unheated comparisons, the average survival was greatest in the groups fed heated fats although the differences were not statistically significant (Table III). When data for both sexes and all fats are combined the average survival periods for the heated and unheated fat-fed animals are almost identical, i.e., 688 vs. 692 days.

There is a substantial indication that the subgroups fed diets containing S or HS survived longer than the subgroups fed the other fats, although the differences are not statistically significant at the $P \leq 0.05$ level. The lard-fed rats were the least consistent in their responses to the treatments, the males fed heated lard surviving longer than those fed unheated lard, whereas the females reversed this order. Furthermore, the lard-fed males had the shortest average survival period of all the male

TABLE IV

Incidence of Tumors in Rats Fed Throughout Life Diets Containing Different Fats

Type fat	Number of rats fed[a]	Percent of animals showing tumors				
		Females			Males	
			Nonmammary		Nonmammary	
		Mammary	Benign	Malign.	Benign	Malign.
CS	9	44	22	11	33	0
HCS	24	50	4	4	12	8
CO	9	22	0	33	0	22
HCO	24	50	4	13	4	21
L	9	56	11	11	0	11
HL	24	46	0	13	8	13
S	9	45	11	0	0	0
HS	24	46	0	17	4	4
Totals						
Unheated	36	42	11	14	8	8
Heated	96	48	2	12	7	12

[a]For each sex.

subgroups. At least part of the shorter survival of the unheated lard-fed males may be attributed to unexplained early deaths of three of the nine-member life-survivor subgroup. The expected longer survivals of all female subgroups in comparison with the corresponding male subgroups is highly significant ($P \leq 0.01$).

Gross Observations at Autopsy: Organ Weights

The detailed gross observations made at autopsy were distributed rather uniformly among the groups with no unusual observations or conditions attributable to heating of the fats.

The consumption of diets including heated fats did not result in significantly different kidney or adrenal weights when compared with organs from animals fed unheated fats. For those rats routinely killed for tissue samples during the first year of the experiment, the liver-body weight ratios were higher for animals fed heated fat diets than for those fed the corresponding unheated fat diets for all comparisons except that for the S-HS male subgroups. The increases were significant ($P \leq 0.05$) for the male and female subgroups fed the HCS diet, and for the female subgroup fed the HL diet. No significant differences attributable to the heating of the fat components of the diets were found for livers removed from rats found dead or dying, but the liver weights and the liver-body weight ratios of male rats fed the HCS diet were significantly heavier ($P \leq 0.05$) than those for male rats fed diets containing HCO, HL or HS.

Histopathology

Pathological changes were those commonly found in old animals and included respiratory disease, chronic nephritis and periarteritis, the latter occurring mostly in males without relation to diet. In the animals with tumors, the incidence of nephritis was low in those fed hydrogenated vegetable oil (less than 8% compared to 21% on the other diets and present whether fats were heated or unheated).

Table IV gives the incidence of tumors in both sexes and characterizes them as benign or malignant. Of 132 male rats, 23 had tumors, of 132 female rats, 77 were tumor bearers, 61 had mammary tumors and 5 of these had additional tumors located elsewhere.

Mammary tumors were found in 46% of the female rats with a similar incidence among the groups fed different fats. Because of their superficial location they were observed early and were mostly of long duration. The average age at death for mammary tumor bearers varied from 728 days for those fed corn oil to 797 days for those fed hydrogenated vegetable shortening. Tumor weights varied greatly; 15 were under 50 g and 25 were over 250 g. The largest tumor, weighing 835 g, was found in a rat dying at 539 days and had been noted first at 296 days. The body weight after the tumor was removed was 242 g. This was the youngest rat of those dying with mammary tumors. Mammary tumors were often multiple and some were bilateral. The usual picture was that of a benign fibroadenoma. Some showed chronic inflammatory reaction and occasionally abscess formation. Adrenal enlargement was associated with these tumors, of 33 adrenals examined, 24 showed extensive hemorrhage often with cortical lipoidosis. Nonmammary tumors were as frequent in males as in females.

Relatively few tumors developed with diets containing hydrogenated vegetable shortening. Benign tumors predominated in the rats fed cottonseed oil. The highest incidence of malignancy was observed in the animals fed corn oil but too few tumors were involved to establish the significance of these findings. Neoplasms in these rats were mainly of connective tissue origin (sarcomas) rather than of epithelial tissues (carcinomas). Skin tumors, five in male rats and two in females were listed as epitheliomas since they showed excessive growth of cells, formation of keratinized epithelial pearls and local infiltration, but were of low malignancy and probably secondary to chronic ulceration. No distant spread was present except in one 928 day old female with a large tumor between the legs which had some nodules in the mesentery. Subcutaneous tumors were found in nine males and five females. In the male, seven were benign and two sarcomatous. In the female all but one were malignant, of these one was carcinomatous. An adenocarcinoma of the salivary gland might also be added to this group. In none of these were distant metastases found. Abdominal tumors, including those of the gastrointestinal, endocrine and reproductive systems, were found in 10 males and 14 females. In male rats, three were benign and seven malignant, mostly diffuse and involving the gastrointestinal tract; five of these were sarcomatous and two carcinomatous. In female rats there were five benign and nine malignant tumors. The benign tumors included two adenomas of the adrenal gland, two of the pancreas and one uterine fibromyoma. In contrast to males, tumors of the reproductive system were common, six occurring in the uterus and adnexas of which five were malignant. A pancreatic duct carcinoma was the only tumor with distant metastases to the lung. The only primary tumor in the liver was a bile duct carcinoma. Two diffuse lymphosarcomas were found involving most of the abdominal viscera.

Age Incidence of Nonmammary Tumors

The average age at death for males with benign or malignant tumors was over 675 days. Female rats with benign tumors averaged 691 days, those with malignant tumors 546 days. The earlier deaths occurred mostly in those with tumors of the reproductive system.

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METABOLIZABLE ENERGY VALUES OF FATS AND FATTY ACIDS FOR CHICKENS

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Studies on the metabolizable energy content of various fats and fatty acids have been conducted during the past year as part of a general program in this laboratory to determine the energy values of purified nutrients and practical feedstuffs for the chicken.

Metabolizable energy was determined by bomb calorimetry using the chromic oxide indicator method and correcting for nitrogen retention.[1] Metabolizable energy values for various fats and fatty acids were obtained by feeding chicks semi-purified diets in which the given fat was substituted either weight for weight or isocalorically for glucose (Cerelose). From the difference in metabolizable energy content of the reference diet and fat-containing diets, metabolizable energy values for fats and fatty acids were determined, assuming the metabolizable energy value of glucose, the standard reference substance, to be 3.64 Cal. per gram on a dry matter basis.[2]

By comparing the metabolism energy value of fat to its gross energy, a measure of fat utilization is obtained. Such values reflect not only the utilization of fat itself, but also the effect of fat on the digestibility of other components of the diet. More usually fat utilization has been studied by digestibility, from which a digestibility coefficient is determined by comparing the amount of fat excreted to the amount ingested after correcting for metabolic fat. In the present work, simultaneous determinations of fat utilization by the two methods were used. They were generally in good agreement, but showed that utilization expressed as metabolizable energy was slightly less than the digestibility of the fat. Three possible explanations can be postulated for the difference between the two methods. Either, (a) the high level of fat adversely affected the utilization of some other nutrient in the diet, or (b) the metabolizable energy value used for the reference substance (glucose) may be slightly low. On the other hand, (c) digestibility coefficients could be erroneously high if determination of fecal fat is incomplete.

Summarized in Table 1 are the metabolizable energy

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values of the various fats and fatty acids studied together with their utilization as indicated by metabolizable energy as a percentage of gross energy.

All values listed in Table 1 were obtained with fat or fatty acid included in the diet at a level of approximately 20%. Anderson[3] found no difference in the metabolizable energy obtained for stabilized tallow when fed at levels of 10 and 20% of the diet. He reported values of 3260 and 3240 Cal/lb. respectively. In the case of other feedstuffs as well, level of substitution has not affected determination of the energy value, except for the readily predictable relationship that precision increases with higher levels of substitution.

The metabolizable energy values of the various fats and fatty acids represent values for the chick at 3-4 weeks of age except in the case of feed grade tallow, lard and corn oil where a systematic study was made to determine their utilization at 2, 4, 6 and 8 weeks of age.[1] Results of this study showed that only the utilization of tallow was affected by the age of the chick, older chicks being better able to utilize tallow than younger chicks. Lard and corn oil were equally well utilized by all age groups.

Of the edible fats studied, soybean oil had the highest energy value for the chick while corn oil and lard were also very well utilized. Hydrogenated vegetable fat, although more unsaturated than lard, was less well utilized.

Among the inedible fats, the fish oils had the highest energy value, however, they were less well utilized than either soybean oil, corn oil or lard. On the average, fish oils were utilized to 86% which is in marked contrast to the human, rat and guinea pig which utilize cod liver oil to 98%, 98% and 94% respectively.[7] The two samples of tallow had the lowest energy value in this group of fats, ranging from 2860 to 3000 Calories per pound. Pure beef tallow had a lower energy value than feed grade tallow for the four week old chick, which was to be expected since the latter contains some pork fat, which is better utilized by the chick than beef tallow. Hydrolyzed fat (a feed grade fat composed of approximately equal parts of fatty acids and neutral fat from animal and vegetable sources), acidulated soybean soapstock and soybean lecithin had slightly higher energy values than the tallows.

In an effort to gain information on fat digestion and absorption in the chick, fatty acid mixtures derived from beef

tallow, lard and soybean oil were compared with the respective intact fats. The energy values of the mixtures of fatty acids were much lower than the energy values of the respective neutral fats. From the composition data for these mixtures it appears that the free saturated fatty acids were not utilized even in the presence of unsaturated fatty acids. This is consistent with the finding that stearic and palmitic acids when fed singly are not utilized by the chick at 4 weeks of age while oleic acid is utilized to about 88%.[6] Sunde[7] has also reported that stearic acid is poorly utilized by the chick.

The metabolizable energy value obtained for a fat depends on two physiological processes, digestion and absorption. Since Mattson[8] discovered that pancreatic lipase is specific for reactions occurring at the primary hydroxyl groups of triglycerides, a relatively clear picture of the mechanism of fat digestion in the rat is available; however, the process of fat absorption is still obscure. According to the lipolytic theory, fat is completely hydrolyzed in the intestinal tract to free fatty acids and glycerol. According to the partition theory, digestion proceeds only far enough to yield sufficient monoglycerides and free fatty acids which together with the bile salts emulsify the remaining triglycerides. According to this theory, the main bulk of fat is absorbed as the triglyceride. Recently Reiser[9] has concluded from experiments using radioactive techniques that only 3% of tripalmitin was absorbed unhydrolyzed by the rat. He postulated that fat is broken down to free fatty acids and monoglycerides which are resynthesized in the intestinal mucosa to form neutral fat.

The poor utilization of free fatty acids which was obtained in these studies supports the theory that either neutral fat or some intermediate breakdown product of triglycerides is required for absorption of free fatty acids. Determination of the component or components of neutral fat which are required awaits further study.

Up to recent years, two theories have been used to attempt to explain differences in digestibility of intact fats. Crockett and Deuel[10] and Langworthy[11] postulated that digestibility is inversely proportional to and dependent on melting point, while Hoagland and Snider[12] and Mattil[13] postulated that digestibility is limited by the amount of saturated fatty acids of carbon chain 18 or longer. Application of either of these theories to the fats studied does not explain the similar utili-

zation of lard and corn oil by the chick, the large difference in utilization of lard and tallow, or the differences in utilization of soybean oil, corn oil and fish oils. Recently, however, Calloway and coworkers[11] have concluded that digestibility is primarily dependent on the amount and chain length of saturated fatty acids and their arrangement within the glyceride structure. Thus, further studies may show that the difference in digestibility of lard and tallow which cannot be explained entirely by their saturated fatty acid content or melting point is related to the arrangement of fatty acids in the glyceride molecule. Similarly, such studies may reveal why lard containing 35% saturated fatty acids is as well utilized as corn oil containing 11% saturated fatty acids.

SUMMARY

Metabolizable energy values of several well characterized fats, mixtures of fatty acids and individual samples of oleic, palmitic and stearic acid have been determined for the chick. Soybean oil, corn oil and lard were of high energy value for the chick. A sample of hydrogenated vegetable fat, although more unsaturated than lard, was less well utilized. Of the inedible fats or fat by-products studied, fish oils had the highest energy value, hydrolyzed fat, acidulated soybean soapstock and soybean lecithin were intermediate, while beef tallow had the lowest energy value. Results indicate that some factor in addition to melting point and saturated fatty acid content determines the relative metabolizable energy values of various fats.

Mixtures of fatty acids had much lower energy values than the neutral fats from which they were derived, supporting the theory that only partial hydrolysis of fat occurs in the digestive process. Composition data indicate that saturated fatty acids in such mixtures are not utilized. This is consistent with the finding that stearic and palmitic acids when fed singly are not utilized by the chick.

The utilization of fats and fatty acids measured by metabolizable energy value agreed closely with digestibility coefficients determined simultaneously. This shows that the inclusion of fat in the diet had no major effect on utilization of other components.

TABLE 1. Metabolizable energy values and utilization of fats and fatty acids

	Iodine number	M.E. Cal./lb.	Utilization %
Edible fats			
Corn oil	122	3950	92.9
Degummed soybean oil	133	4210	98.8
Lard	65	3980	93.4
Hydrogenated fat	69	3250	76.2
Inedible fats			
Feed grade tallow	52	2990**	70.3
		3230***	76.2
Beef tallow	44	2860	67.0
Hydrolyzed fat	61	3230	77.8
Menhaden oil	169	3700	87.8
Red fish oil	120	3580	83.4
Cod liver oil	154	3690	87.0
Fatty acids			
Palmitic		−9	−0.2
Stearic		−17	−3.9
Oleic		3770	88.4
Soybean F.A.	131	3750	89.3
Lard F.A.*	67	2730	64.8
Tallow F.A.*	44	2010	47.7
Miscellaneous			
Acidulated soybean soapstock	124	3150	75.3
Soybean lecithin		3060	86.5

* Derived by hydrolysis from the samples of beef tallow and lard studied.
** Young chicks, 2-4 weeks old.
*** Older chicks, 6-8 weeks old, and hens.

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The Utilization of Corn Oil, Lard and Tallow by Chickens of Various Ages

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QUANTITATIVE data on the utilization of fats and fatty acids by poultry are quite limited. Whitson *et al.* (1943) described an index method for determination of digestibility using barium sulfate, and showed that soybean oil is highly digested and absorbed by growing chickens. Duckworth *et al.* (1950) found that linseed oil is well utilized by chicks, but mutton tallow is much less well absorbed. Their data indicated that the absorbability of mutton fat increased with age. Carver *et al.* (1955) found that beef tallow was well utilized when added at a level of 3% to a practical broiler ration. Hydrogenated tallow and the fatty acids of hydrogenated tallow were less well absorbed, while oleic acid showed high absorbability. Sunde (1956) obtained evidence that the hard fats, tallow and hydrogenated tallow, when fed at a level of 5% in a practical chick ration were less well utilized than softer inedible fats classified as greases. Stearic acid was poorly absorbed in his work, while oleic, linoleic, and linolenic acids were highly absorbed. March and Biely (1957) showed large differences in apparent absorbability between corn oil (85–92%), beef tallow (73–75%) and hydrogenated fat (50%) when fed to growing chicks.

The studies to be described were conducted to determine the utilization of corn

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oil, lard and tallow by chickens of different ages. Two methods for studying fat utilization were employed: (1) metabolizable energy value determined by bomb calorimetry, and (2) determination of absorbability by analysis of diets and excreta for fats and fatty acids. The purpose of using both methods to estimate fat utilization was to compare their usefulness, and to determine whether the fats affected the utilization of other dietary constituents.

MATERIALS AND METHODS

One experiment was conducted with male crossbred (RIR × BPR) chicks. They were randomly distributed into lots of 10 chicks each and housed in electrically heated, thermostatically controlled battery brooders with raised wire screen floors in a temperature controlled laboratory. Two duplicate groups were fed each of the experimental diets, and feed and water were supplied *ad libitum*. Another experiment was conducted with adult laying hens. Single Comb White Leghorns, 9 months old, were used for this purpose. Two duplicate groups of two hens each, maintained in wire-floored layer cages in a heated laboratory room, were used for each dietary treatment.

The composition of the experimental diets is shown in Table 1. The low fat reference diet was the same as diet E9 described in detail by Hill *et al.* (1960). Preliminary work had indicated that tallow had a metabolizable energy for chicks of approximately 7.7 Calories per gram, so the fats were incorporated in the respective diets on the assumption that they all had approximately this value. On this basis, 17.5 parts of each of the respective fats replaced 36.9 parts of glucose on a dry matter basis to achieve approximately equicaloric substitution of fat for carbohydrate, using the value 3.64 Calories per gram of glucose previously determined in this laboratory (Anderson, Hill and Renner, 1958). Corn oil (Mazola) and lard were obtained through food distribution channels. The tallow was a feed grade material composed of approximately 80% beef tallow and 20% inedible pork fat.

TABLE 1.—*Composition of experimental diets*

	Parts in	
	Low-fat reference diet	Fat supplemented diets
Constant components[1]	56.6	56.6
Glucose (Cerelose)	43.4	6.5
Fat (corn oil, lard, or tallow)	—	17.5
Total parts	100.0	80.6

[1] Ground wheat 9, dehulled soybean oil meal 15.8, crude casein 10.5, gelatin 2.5, fish meal (menhaden) 4, fish solubles 2, dried brewers yeast 2.5, dried whole whey 2, hydrogenated vegetable shortening 2.5, BHT .01, DPPD .01, mineral supplements 4, vitamin mixture .8, chromic oxide "bread" 1. Composition of mineral and vitamin supplements and chromic oxide "bread" described in detail by Hill, Anderson, Renner and Carew (1960).

During the course of the experiment with growing chicks, excreta were collected during the first four days of the second, fourth, sixth and eighth week of life. In the experiment with hens, excreta collections were made after they had been fed the experimental diets for 1 week. Chromic oxide was incorporated in each of the diets at a level of approximately 0.3% as an index substance in order to establish the amount of excreta produced per unit of feed intake without need for quantitative collection of the excreta or quantitative measurement of feed intake. The method of processing excreta, conducting chemical analyses for moisture, nitrogen, combustible energy, and chromic oxide, and the method of computing metabolizable energy of the diet from these data, have been described previously in detail (Hill and Anderson, 1958; Hill, Anderson, Renner and Carew, 1960). The metabolizable energy of each of the three fats was computed a

follows: the metabolizable energy per gram of the low fat reference diet minus the metabolizable energy of the glucose replaced by fat (3.64 × 0.369) gave the calculated value of the constant portion of the diet (0.631 grams); the metabolizable energy per 0.806 grams of fat-supplemented diet minus the metabolizable energy of 0.631 grams of constant portion gave the metabolizable energy of 0.175 grams of the respective fat, from which the value per gram was derived. The utilization of each fat was computed from its metabolizable energy value as a percentage of its gross combustible energy using the determined values 9.44, 9.42 and 9.34 Calories per gram for corn oil, lard and tallow, respectively.

Absorbability of the various fats was determined by appropriate analysis of diets and excreta for fats and fatty acids. Dietary fat was determined by ether extraction in a Goldfisch extraction apparatus. The total fat excreted, including neutral fat and fatty acids, was determined by the following modification of the method of Fowweather and Anderson (1946):

> A two gram sample of dried feces ground to pass a 40 mesh screen was mixed with 3 cc. water and 1 cc. concentrated HCl in a small mortar and left for 30 min. Six grams anhydrous $MgSO_4$ was then added and the ground mixture allowed to stand overnight in an oven at 40°C. The mixture was then extracted for 3 hrs. with petroleum ether (60°–70°C.) in a Goldfisch apparatus after which excess solvent was removed in a steam bath and the extracts dried for 30 minutes in a forced air oven at 105°C.

Correction for endogenous fat and fecal fat contributed by constant diet ingredients was made from the data obtained for the low fat reference diet. Because the level of chromic oxide was essentially identical in all diets in respect to the non-glucose, non-fat ingredients, the fat excretion per unit of index substance on the low fat reference diet could be used to estimate endogenous and basal unabsorbed fat. This estimate was subtracted from the fat excreted per unit of chromic oxide by the groups fed the fat supplemented diets. The correction was of the order of 3–5% of the dietary fat for chicks and 2% for hens at the level of supplementation employed in this experiment. Absorbability was computed from the corrected fat excretion per unit of food intake as compared to the level of the respective fat contained in the diet.

At the completion of the experiment, the combined data on the utilization of all fats by the chick were subjected to an analysis of variance (Snedecor, 1956). The data for the hens were analyzed in the same way.

RESULTS AND DISCUSSION

Summarized in Table 2 are data showing the utilization of corn oil by growing chicks of various ages and adult hens. Analysis of variance (Snedecor, 1956) showed that the metabolizable energy value of corn oil for chicks did not change significantly in the course of the experiment from two to eight weeks of age. Expressed as a percentage of gross combustible energy, the metabolizable energy value of corn oil ranged from 91–96% for chicks and was 90% for the adult hen. Also shown in Table 2 are data on the absorbability of corn oil, based on determinations of fat and fatty acids in the diets and excreta. Percentage utilization by this technique ranged from 94–98% for chicks and was 94% for adult hens.

Similar data on the utilization of lard are shown in Table 3. Utilization as determined by metabolizable energy value ranged from 89–92% for chicks in the period from two to eight weeks of age, and no change with age was evident over this period. A lower metabolizable energy value

TABLE 2.—*Utilization of corn oil by chickens of various ages*

Age in weeks	Energy utilization: Metabolizable energy		Energy utilization: Percent of gross energy[1]		Absorbability[2]	
	Cal./gm.				%	
2 weeks	8.89		94		97	
	8.36	8.62[3]	89	91	92	94
4 weeks	8.94		95		98	
	9.15	9.04	97	96	98	98
6 weeks	8.91		94		97	
	8.50	8.70	90	92	96	97
8 weeks	8.69		92		95	
	8.72	8.70	92	92	95	95
38 weeks (adult hens)	8.56		91		95	
	8.42	8.49	89	90	94	94

[1] Metabolizable energy as a percentage of gross energy of corn oil (9.44 Cal./gm.).
[2] Computed from dietary fat and total fecal fat, correcting for fecal fat from low-fat reference diet.
[3] Underlined values are averages of duplicate lots; individual replicate values are in the respective left columns.

equal to approximately 82% utilization of gross energy was shown by the adult hen. That the lower metabolizable energy value shown by hens was due to reduced absorption of the fat is shown by the data for absorbability. While the chicks absorbed lard to the extent of 90–95%, the hens showed only 85% utilization by this measure.

The observation that the utilization of lard by the hen was less efficient than by the growing chick was quite unexpected. In subsequent work using a different source of lard, hens have shown a metabolizable energy for lard equal to that obtained with chicks. However, no ready explanation for the difference found in the present experiment is apparent.

Data on the utilization of tallow by chicks and hens are shown in Table 4. In contrast to the absence of any trend in fat utilization with age of chicks for other fats, the utilization of tallow increased with age in the range from two to eight weeks of age. Utilization as measured either by metabolizable energy or absorbability showed that the chick eight weeks of age is equal to the hen in efficiency of utilization of this type of fat.

The increase in tallow utilization as the chick grows older is somewhat similar to the data reported by Duckworth *et al.* (1950), and described above. Bose and Subrahmanyan (1950) also noted a similar improvement with age in the digestibility of peanut oil and the saturated fatty acids from hydrogenated peanut oil in the rat. Norcia and Lundberg (1954) found that the utilization of tripalmitin by the rat was increased with time.

TABLE 3.—*Utilization of lard by chickens of various ages*

Age in weeks	Energy utilization: Metabolizable energy		Energy utilization: Percent of gross energy[1]		Absorbability[2]	
	Cal./gm.				%	
2 weeks	8.69		92		91	
	8.55	8.62[3]	91	92	90	90
4 weeks	8.30		88		91	
	8.50	8.40	90	89	92	91
6 weeks	8.46		90		93	
	8.81	8.63	94	92	96	95
8 weeks	8.67		92		92	
	8.72	8.70	93	92	92	92
38 weeks (adult hens)	7.81		83		86	
	7.72	7.76	82	82	85	85

[1] Metabolizable energy as a percentage of gross energy of lard (9.42 Cal./gm.).
[2] Computed from dietary fat and total fecal fat, correcting for fecal fat from low fat reference diet.
[3] Underlined values are averages of duplicate lots; individual replicate values are in the respective left columns.

TABLE 4.—*Utilization of tallow by chickens of various ages*

Age in weeks	Energy utilization: Metabolizable energy		Energy utilization: Percent of gross energy[1]		Absorbability[2]	
	Cal./gm.				%	
2 weeks	6.18 6.97	6.58[3]	66 75	70	65 74	70
4 weeks	6.45 6.69	6.57	69 72	70	74 74	74
6 weeks	7.15 6.69	6.92	77 72	74	80 70	75
8 weeks	7.46 7.18	7.32	80 77	78	83 80	82
38 weeks (adult hens)	7.15 7.23	7.19	77 77	77	80 82	81

[1] Metabolizable energy as a percentage of gross energy of tallow (9.34 Cal./gm.).
[2] Computed from dietary fat and total fecal fat, correcting for fecal fat from low fat reference diet.
[3] Underlined values are averages of duplicate lots; individual replicate values are in the respective left columns.

Comparison of the two methods for studying fat utilization showed that metabolizable energy and absorbability were quite comparable for chicks fed lard or tallow. However, statistical analysis showed that values for the utilization of corn oil by chicks and of all three fats by hens were slightly but significantly higher when determined by absorbability.

Three possible explanations to account for differences between the two methods can be advanced. It is possible that the value 3.64 Calories per gram glucose dry matter used as the reference standard may be slightly lower than its true value. In this case, any adjustment in the value of the reference standard would result in a corresponding change in the value of substances measured against it. Secondly, the high level of fat employed might have had an adverse effect on the utilization of some other component of the diet; this would have the effect of reducing the apparent metabolizable energy value of fat while the absorbability measurement would be unaffected. It is also possible that the method used to determine fat in the excreta failed to extract all of the fecal lipids. In this case the method would underestimate the unabsorbed fats and therefore overestimate the true absorption.

In the case of the hen it is not possible to choose among these three explanations. In the chick, however, since the values obtained for utilization of lard and tallow by the two methods were the same, it is unlikely that the energy value of glucose is contributing to the difference between methods in the utilization of corn oil. However, the discrepancy was relatively small for both chicks and hens, and it is evident from the data presented that metabolizable energy value and absorbability of the respective fats are closely related.

Any effect of high dietary fat levels on the digestion and absorption of other nutrients by the chicken is evidently small so far as over-all energy utilization is concerned. If the discrepancy discussed above is due to such an effect, its direction is negative since the metabolizable energy value of fat gave an index of utilization which was slightly lower than its apparent absorbability.

SUMMARY

The utilization of corn oil, lard and tallow by growing chicks of various ages and by adult hens has been studied by determining the metabolizable energy value of the fats and their absorbabilities.

Age affected only the utilization of tallow by the growing chick. In early life, utilization of this fat was lower than in the adult. By the time the chick was eight weeks of age it was able to utilize tallow

equally as well as the adult hen.

Metabolizable energy and absorbability were comparable for chicks fed lard and tallow. Values for the utilization of corn oil by chicks and for corn oil, lard and tallow by hens were slightly higher when determined by absorbability. From the size and nature of the difference, it appears that a high dietary level of fat does not have any major effect on the digestion and absorption of other nutrients, and whatever slight effect may occur is negative.

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The Influence of Varying Levels of Dietary Protein, Carbohydrate and Fats in the Nutrition of the Rat[1]

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Dietary levels of protein, carbohydrate and various fats in animal nutrition have received considerable attention in the last decade, with respect to their effect on lipid metabolism. Previous reports have indicated that the substitution of unsaturated fats for saturated fats in the diet tends to lower serum cholesterol and to increase liver cholesterol (Aftergood et al., '57; Alfin-Slater et al., '54). It has been suggested that unsaturated fats facilitate the transport of cholesterol to the liver for excretion as bile salts (Holman, '56).

The effects of different levels of dietary protein and of protein quality on lipid metabolism have been investigated in experimental animals. Kokatnur et al. ('58) reported that increased levels of dietary protein were associated with decreased serum cholesterol levels in the chick, however, Lushbough et al. ('60) and Jones and Huffman ('56) observed that long-term feeding of high levels of dietary casein tend to increase serum cholesterol levels in the normal rat. Seidel et al. ('60) reported that the addition of either protein or sulfur amino acids to a low protein diet will decrease serum cholesterol in the hypercholesterolemic rat.

In the long-term feeding studies reported here, the responses of male albino rats fed varying dietary levels of protein, carbohydrate and fats were evaluated. Feed and caloric efficiencies and rate of weight gain were determined in young rats and measurements of the total serum cholesterol, total liver cholesterol and total serum polyunsaturated fatty acid levels were made in the young adult and adult rat.

EXPERIMENTAL METHODS

Male weanling rats[5] were fed a stock diet[6] for three days and the animals were then allocated by weight into 9 experimental groups. Each group consisted of 14 animals. The animals were housed in individual cages with raised screen floors. Feed and water were provided ad libitum until the animals reached a weight of 500 gm, whereupon the feed intake was regulated to maintain their weight as closely as possible at 500 gm. The animals were weighed each week throughout the 57-week experimental period. Feed intake was measured during the first 4 weeks of the experiment. Feed and caloric efficiencies were determined during this initial 4 week feeding period. The compositions of the diets used in this study are shown in table 1.

Blood samples were obtained from all animals after 6 weeks on experiment. At this time, three animals were chosen at random from each of the 9 groups and killed by terminal aortic bleeding (Lushbough et al., '61). The aorta and heart from each of the sacrificed animals were fixed in formalin prior to histological determinations of abnormal fat depositions. Individual livers obtained from each sacrificed animal were analyzed for total cholesterol. Blood samples were obtained by tail vein bleeding from the remaining ani-

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TABLE 1

Composition of experimental rations

Diet no.	Casein		Corn oil[1]		Lard[2]		Sucrose		Basal fat[3]		Salts IV[4]	Vitamin mix[5]	Cal./100 gm[6]
	Weight	Cal.	Weight	Cal.	Weight	Cal.	Weight	Cal.	Weight	Cal.	Weight	Weight	
	%	%	%	%	%	%	%	%	%	%	%	%	
1	19.8	20	—	—	—	—	73.2	74	2.7	6	4.0	0.3	396
2	33.4	20	—	—	54.9	74	—	—	4.5	6	6.7	0.5	668
3	33.4	20	54.9	74	—	—	—	—	4.5	6	6.7	0.5	668
4	49.5	50	—	—	—	—	43.5	44	2.7	6	4.0	0.3	396
5	65.3	50	—	—	25.5	44	—	—	3.5	6	5.3	0.4	523
6	65.3	50	25.5	44	—	—	—	—	3.5	6	5.3	0.4	523
7	79.2	80	—	—	—	—	13.9	14	2.6	6	4.0	0.3	396
8	85.8	80	—	—	6.7	14	—	—	2.9	6	4.3	0.3	429
9	85.8	80	6.7	14	—	—	—	—	2.9	6	4.3	0.3	429

[1] Mazola, Corn Products Company, Argo, Illinois.
[2] Unstabilized lard.
[3] A mixture of equal weights of corn oil and lard flakes.
[4] Hegsted et al. ('41).
[5] The following vitamins were added per 100 Cal. of the ration: (in milligrams) thiamine·HCl, 0.154; riboflavin, 0.154; nicotinic acid, 0.513; folic acid, 0.051; Ca pantothenate, 1.026; biotin, 0.002; pyridoxine·HCl, 0.154; choline chloride, 38.7; inositol, 25.6; p-aminobenzoic acid, 7.7; vitamin B_{12} (0.1% trituration), 0.513; menadione, 0.8; vitamin A acetate, 0.36; calciferol, 0.002; and DL-α-tocopherol, 1.28.
[6] Calories were calculated as 4 Cal./gm for casein and sucrose, 9 Cal./gm for lard and corn oil.

mals. The same procedures of bleeding and obtaining terminal blood, liver, heart and aorta samples were used at the end of 14 and 22 weeks. After 57 weeks, the 5 animals remaining in each group were killed and the analytical determinations completed.

Individual serum and liver cholesterol levels were determined using the method of Abell et al. ('52). Total polyunsaturated fatty acid content of individual serum samples was determined using the lipoxidase method of MacGee ('59). Histological sections of the hearts and aortas were stained with Sudan IV and the stained sections were examined microscopically for gross evidence of abnormal fat deposits.

RESULTS AND DISCUSSION

The average weight gains, feed and caloric efficiencies of the test groups after feeding the experimental diets for 4 weeks are shown in table 2. Average weight gains were greatest for the animals fed the diets containing 50% of their calories as casein (diets 4, 5 and 6) and the low casein-lard ration (diet 2). The animals fed 80% of their calories as casein (diets 7, 8 and 9) had the smallest average weight gains and those fed the low casein-sucrose and low casein-corn oil diets (diets 1 and 3) were intermediate in weight gains after the initial 4 weeks.

Feed efficiencies, expressed as grams of gain per gram of feed intake, increased when either lard or corn oil was substituted for sucrose at each of the casein levels tested.

When 20% of the calories were supplied as casein (diets 1–3), the substitution of lard for sucrose resulted in a decreased caloric efficiency of feed utilization as compared with either the sucrose or corn oil diets. The reasons for the relatively low caloric efficiency observed in these animals fed diet 2 cannot be explained. When the calories supplied by casein were increased from 20 to 50% in the diet, the caloric efficiency of feed utilization was markedly increased. When 80% of the calories were supplied by casein, however, decreased caloric efficiencies were observed for the sucrose and corn oil groups as compared with the re-

spective groups fed 50% of the calories as casein. In the groups fed 50% or 80% of their calories as casein, substitution of either lard or corn oil for sucrose increased the caloric efficiency values. Similar results were obtained in rats and chicks by Beilharz and McDonald ('59), Yoshida et al. ('58) and Lushbough and Schweigert ('60).

Whereas most of the experimental groups required food restriction to maintain a weight near 500 gm throughout the experiment, groups fed the high protein diets (80% of their calories as casein, diets 7–9) required very little, if any, food restriction during the maintenance period. Whether this self-imposed weight regulation in the groups fed the high level of protein is due to increased satiety, poor palatability or an actual difference in energy metabolism is currently under study.

The serum cholesterol values obtained after feeding the experimental diets for 6, 14, 22 and 57 weeks are shown in table 3. There were no apparent differences in the average serum cholesterol values between any of the groups during the first 22 weeks.

TABLE 2

Effects of dietary treatment on the average body weight gain, feed and caloric efficiency of weanling rats over the initial 4-week test period[1]

Treatment[2] Diet no.	Percentage of Cal./ ingredient	Average gain/gm feed consumed	Average gain/100 Cal. consumed	Average gain in body weight[3]
		gm	*gm*	*gm*
1	20/casein, 74/sucrose	0.38	9.6	179 ± 14
2	20/casein, 74/lard	0.56	8.4	196 ± 15
3	20/casein, 74/corn oil	0.67	9.5	182 ± 9
4	50/casein, 44/sucrose	0.43	10.8	198 ± 16
5	50/casein, 44/lard	0.59	11.3	197 ± 15
6	50/casein, 44/corn oil	0.70	13.4	191 ± 14
7	80/casein, 14/sucrose	0.37	9.3	153 ± 15
8	80/casein, 14/lard	0.48	11.2	164 ± 12
9	80/casein, 14/corn oil	0.46	10.7	161 ± 12

[1] Each dietary group consisted of 14 rats.
[2] See table 1 for detailed description.
[3] Mean value ± standard deviation of the mean.

TABLE 3

Effect of the length of dietary treatment on the average serum cholesterol levels in the normal rat

Treatment[1] Diet no.	Percentage of Cal./ ingredient	Average total serum cholesterol — Time on experiment in weeks: 6[2]	14[4]	22[5]	57[6]
		mg/100 ml serum[3]		*mg/100 ml serum*[3]	
1	20/casein, 74/sucrose	70 ± 27	67 ± 7	52 ± 5	75 ± 29
2	20/casein, 74/lard	57 ± 28	70 ± 20	49 ± 18	110 ± 41
3	20/casein, 74/corn oil	55 ± 26	64 ± 15	37 ± 14	83 ± 32
4	50/casein, 44/sucrose	44 ± 30	63 ± 10	43 ± 10	129 ± 26
5	50/casein, 44/lard	64 ± 28	64 ± 18	73 ± 33	90 ± 33
6	50/casein, 44/corn oil	67 ± 19	69 ± 14	59 ± 16	77 ± 40
7	80/casein, 14/sucrose	78 ± 31	69 ± 32	64 ± 24	149 ± 42
8	80/casein, 14/lard	73 ± 29	76 ± 31	85 ± 31	185 ± 43
9	80/casein, 14/corn oil	72 ± 22	71 ± 16	77 ± 28	107 ± 27

[1] See table 1 for detailed information.
[2] Mean values in mg/100 ml serum ± standard deviation of an individual determination.
[3] Fourteen determinations/group.
[4] Eleven determinations/group.
[5] Eight determinations/group.
[6] Five determinations/group.

At the termination of the experimental period (57 weeks), increased levels of dietary casein tended to increase serum cholesterols in the sucrose-fed groups (diets 1, 4 and 7). Animals fed 80% of their calories as casein plus either sucrose or lard (diets 7 and 8) had the highest average serum cholesterol values of any of the test groups. Similar observations that high levels of dietary casein increased serum cholesterol in the normal rat have been reported by Jones and Huffman ('56) and Lushbough et al. ('60). Substitution of lard at the expense of sucrose tended to increase serum cholesterol at the low and high dietary casein levels (diets 1 vs. 2 and diets 7 vs. 8, respectively) and to decrease serum cholesterol values when 50% of the calories were supplied as casein (diets 4 vs. 5). Substitution of corn oil for sucrose decreased the average serum cholesterol of rats fed the diets containing either 50 or 80% of their calories as casein (diets 4 vs. 6 and diets 7 vs. 9, respectively). The groups fed lard had a higher average serum cholesterol than the corresponding groups fed corn oil. The groups fed the lard ration in which 80% of the calories were supplied by casein had the highest average serum cholesterol value of any of the test groups.

The average total liver cholesterol and the average total polyunsaturated fatty acids in the serum of rats fed the experimental diets for 57 weeks are shown in table 4. No consistent differences were noted at 6, 14, or 22 weeks in any of the experimental groups, owing to the limited number of samples (three) obtained from each experimental group at these times. The average liver cholesterol values obtained from the animals fed the experimental diets for 57 weeks (5 samples per group) showed the following trends: at each dietary casein level, the sucrose-fed groups (diets 1, 4 and 7) had the lowest average liver cholesterol; the lard-fed groups (diets 2, 5 and 8) were intermediate; and the groups receiving corn oil at the low and intermediate casein levels (diets 3 and 6) had the highest average liver cholesterol content. As the casein content of the diet was increased, there was a graded decrease in the average liver cholesterol values for the sucrose, lard or corn oil groups. The most marked decreases in average liver cholesterols owing to increased dietary casein were observed in the groups fed corn oil.

The results for the total polyunsaturated fatty acids in the serum of rats fed the test diets for 57 weeks indicate that the high protein-fat groups, both corn oil and lard (diets 8 and 9), had higher average total serum polyunsaturated fatty acid levels than any of the other dietary groups. The significance of the high total serum polyunsaturated fatty acids in the animals fed the high casein-fat diets (diets 8 and 9), as compared with the other experimental groups, cannot be ascertained at this time. The method used here meas-

TABLE 4

Average total liver cholesterol[1] and average total serum polyunsaturated fatty acids[1] of the normal rat after receiving the experimental diets for 57 weeks

Treatment[2] Diet no.	Percentage of Cal./ ingredient	Avg. total liver cholesterol	Avg. total polyunsaturated fatty acids
		mg/100 gm dry weight	*mg/100 ml serum*
1	20/casein, 74/sucrose	532 ± 104	22 ± 10
2	20/casein, 74/lard	698 ± 311	22 ± 5
3	20/casein, 74/corn oil	884 ± 262	29 ± 6
4	50/casein, 44/sucrose	491 ± 171	27 ± 4
5	50/casein, 44/lard	669 ± 184	32 ± 16
6	50/casein, 44/corn oil	781 ± 314	26 ± 14
7	80/casein, 14/sucrose	440 ± 168	25 ± 7
8	80/casein, 14/lard	577 ± 191	45 ± 15
9	80/casein, 14/corn oil	509 ± 140	48 ± 24

[1] Mean values ± standard deviation of an individual determination; 5 determinations/group.
[2] See table 1 for detailed description.

ured total polyunsaturation and did not distinguish between type of fatty acid or polyunsaturation, or both.

The influences of high dietary protein levels on the serum cholesterol, liver cholesterol and total polyunsaturated fatty acids in the serum and the effects of dietary lard as compared with dietary sucrose on the average serum and liver cholesterol in the experimental groups studied do not generally support the cholesterol transport hypothesis. However, the high levels of dietary protein may impair liver function. No differences were noted in the livers on gross observation or in the average weights, (either dry- or wet- weight basis) of the livers from the individual experimental groups. In all instances the ratio between wet and dry liver weights remained relatively constant.

At the termination of the 57-week feeding period, sections of the hearts and aortas of the animals were stained with Sudan IV and examined for evidence of abnormal fat deposits. In no instance was there evidence of abnormal fat deposits in the sections studied.

The studies reported here were designed to evaluate the influence of a wide variation in dietary calorie source on the lipid metabolism of the rat. Individual variation between animals has resulted in a large standard deviation of individual determinations for most of the analytical data obtained. The differences in lipid metabolism owing to dietary treatment are therefore not statistically significant; however, definite trends are indicated. Large numbers of animals and individual analytical determinations within an experimental group are necessary in experiments of this type to allow adequate statistical interpretation.

SUMMARY

The effects of varying levels of dietary protein, carbohydrate and fats on the nutrition of the rat over a 57-week feeding period were studied.

After the rats had received the test diets for 4 weeks, those fed diets containing 50% of their calories as casein had superior rates of gain and utilization of crude calories as compared with rats fed diets containing either 20 or 80% of their calories as casein. Lard or corn oil substituted at the expense of sucrose increased the caloric efficiency of rats fed the diets containing 50 or 80% of their calories as casein.

No apparent differences in average serum cholesterol, average total serum polyunsaturated fatty acids, and average liver cholesterol were noted between the dietary groups through the initial 22 weeks of this study. After 57 weeks, high levels of dietary casein tended to increase serum cholesterol and to decrease liver cholesterol. Substitution of lard at the expense of sucrose tended to increase serum cholesterol at the low and high dietary casein levels, and to increase total liver cholesterol at all dietary casein levels. Substitution of corn oil at the expense of sucrose tended to decrease serum cholesterol at the intermediate and high dietary casein levels and to increase liver cholesterol at all dietary casein levels. The highest liver cholesterol values were obtained in the groups fed corn oil at the lowest dietary casein level. The average total polyunsaturated fatty acids of the serum was increased in the groups fed the high level of casein plus either lard or corn oil.

No evidence of abnormal fat deposits was observed in any of the hearts and aortas from these animals.

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Osteoarthrosis in Mice Fed Diets Enriched with Animal or Vegetable Fat

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In mice of strain C57BL, prolonged feeding of a diet enriched with animal fat accelerated articular aging and accentuated the evolution of osteoarthritis. The intensity of this effect depended upon strain, sex, the age at which the dietary regimen was instituted, and the length of time during which the enriched diet was consumed.[1,2] The high caloric value of the ration as well as the fat specifically were found to be responsible for the altered course of both age changes and joint disease[2] conditions which prevail also in regard to body growth of rats fed fat enriched diets.[3] In continuation of these investigations we have now studied the joints of aging mice fed a diet enriched with a vegetable fat.

Material and Methods

The observations were made on male mice of the closely inbred strain C57BL Jax6 raised in our laboratory. Group A consisted of 77 control animals fed from the time of weaning a stock ration of Purina laboratory chow containing 5% animal fat. Group B consisted of 78 animals fed after weaning the stock ration enriched with 25% lard. Details of the methods used and of the findings in the joints of mice of Groups A and B have been reported elsewhere.[1] Group C consisted of 55 animals kept from the time of weaning on the stock ration enriched with 25% commercial cottonseed oil. This diet was prepared by grinding the laboratory chow to a fine meal and mixing it thoroughly with the oil. Vitamin Diet Fortification Mixture, Nutritional Biochemical Corporation, Cleveland, was added at a 1% level. This diet was well tolerated by the animals. The rations were fed ad libitum, and water was available at all times. The animals were kept under the same environmental conditions as outlined in previous reports.[1] Weights were taken at regular intervals.

The mice were killed in groups at the ages of 18 or 24 months with the exception of those which died during the intervals. Some animals were permitted to live beyond the age of 2 years. Details of the age distribution may be seen from Table 1. At necropsy, femurs and tibias including the knee joints were removed as a whole, fixed and decalcified in Bouin's solution, and embedded in paraffin. Semiserial sections were stained with hematoxylin and eosin. The joints were studied microscopically, and the findings were compared with those obtained in Groups A and B. The results were evaluated statistically with the χ^2 method.

Observations

Weights.—The animals kept on the cottonseed oil-enriched diet gained less weight than those given lard, and their weights

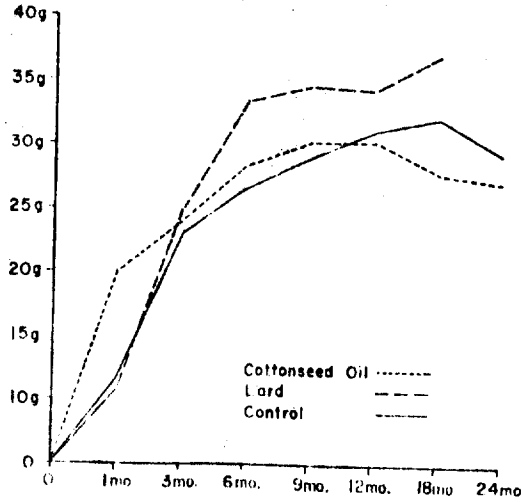

Fig. 1.—Weight curves of mice fed the stock diet, the lard-enriched, and the cottonseed oil-enriched diet respectively.

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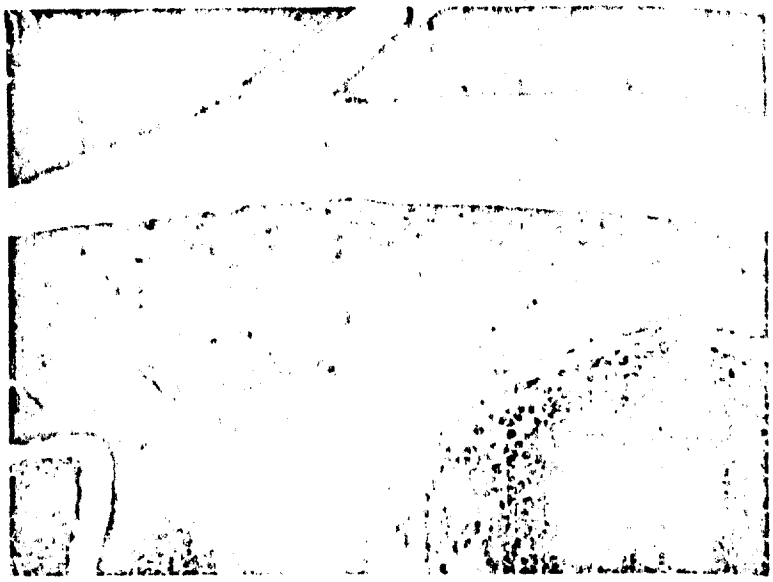
The Figures 2-5 are photomicrographs of articular surfaces.

Fig. 2.—Normal resting joint surface; ×200.



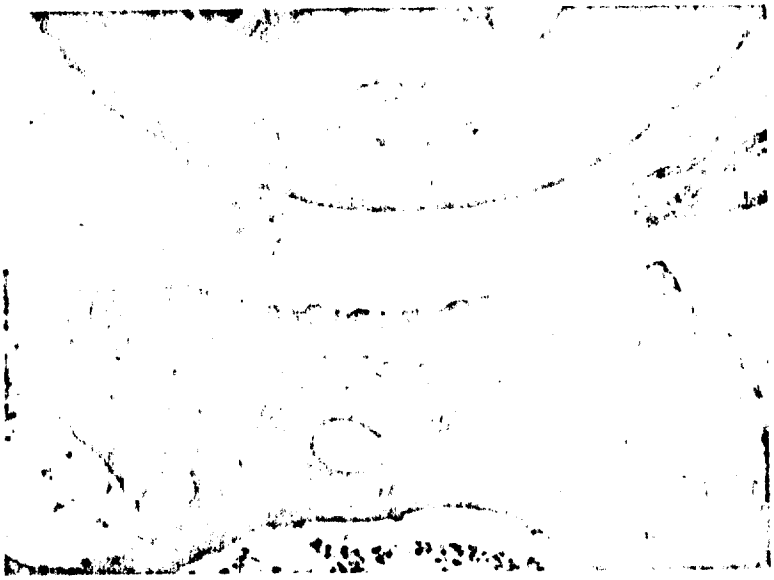


Fig. 3.—Age changes in a joint showing growth and regressive changes with intact surface; ×200.

Fig. 4.—Osteoarthritis with shallow erosion. Femur on top, tibia on bottom; ×50.

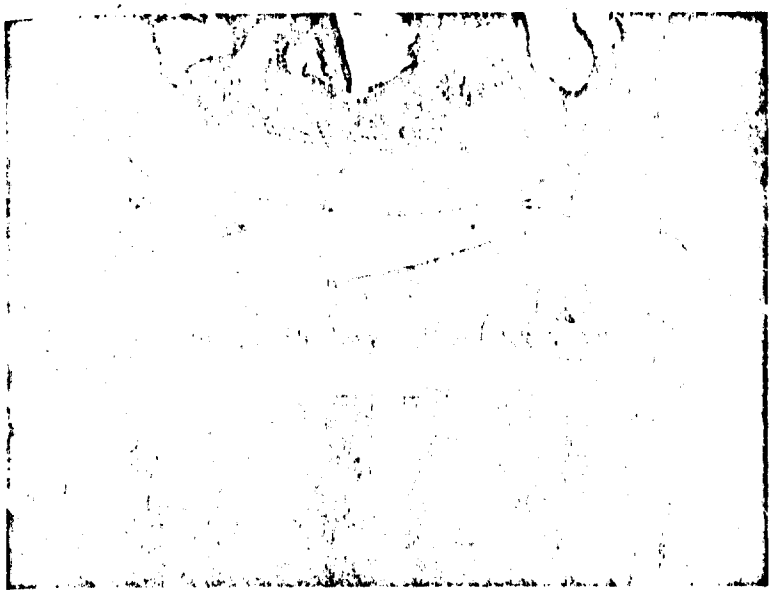


Fig. 5.—Osteoarthritis: ulceration of cartilage and eburnation of underlying bone. Femur on top, tibia on bottom; ×50.

stayed more or less within the limits of the weights of the stock-fed animals. Moreover, mice fed the cottonseed oil diet lived to a relatively old age, and thus failed to show the high mortality observed in mice consuming the lard-enriched diet.[1,4]

Microscopic Examination.—In accordance with criteria fully described before,[1] age changes and osteoarthritic lesions were distinguished. The former are characterized by hyperplasia, hypertrophy and degeneration of cartilage without surface defects. Osteoarthritis may be either hypertrophic or ulcerative or of a combined type and represents an analogue of the human disease.[1] As long as there are slight surface changes such as roughening or shallow erosions, the lesions are considered slight. Severely affected joints disclose large and deep ulcerations, bony outgrowth, involvement of ligaments, synovialis and bone marrow leading to marked deformities. Representative changes are demonstrated in Figures 2-5.

Results.—A compilation of the articular findings is presented in Table 1.

After consumption of the cottonseed oil diet, 3 (14%) of 21 mice had intact joints at the mean age of 16.3 months. Of 24 mice with a mean age of 21.3 months only 1 (4%) showed unchanged articulations, while animals living into the third year of life had either articular age changes or osteoarthritic lesions. The incidence of both remained more or less stationary during the second year of life, the former changing from 48 to 50%, and the latter from 38 to 46%. However, during the third year of life, as the incidence of age changes decreased to

TABLE 1.—*Articular Findings in Mice Kept on a Diet Enriched with Cottonseed Oil*

Age Group, Mo.	Mice No.	Mean Age, Mo.	Articular Findings: No Change No.	No Change %	Age Change No.	Age Change %	Osteoarthritis No	Osteoarthritis %
13-18	21	16.3	3	14	10	48	8 (4) *	38
19-24	24	21.3	1	4	12	50	11 (4) *	46
24+	10	25.2	0	0	3	30	7 (3) *	70
Irrespective of age	55	20.1	4	7	25	46	26 (11) *	47

* Severe osteoarthritis.

TABLE 2.—*Articular Findings in Mice Fed a Stock Diet, a Lard-Enriched and a Cottonseed Oil-Enriched Diet Respectively*

Group	Experiment	Mice No.	Mean Age, Mo.	Articular Findings: No Change		Arc Change		Osteoarthritis	
				No.	%	No.	%	No.	%
A	Stock diet	77	20.3	7	9	37	48	33 (18) *	43
B	Lard diet	78	18.3	3	4	18	23	57 (40) *	73
C	Cottonseed diet	55	20.1	4	7	25	46	26 (11) *	47

( ) * Severe osteoarthritis.

Significance of differences. Incidence of osteoarthritis. Lard-enriched diet: cottonseed oil-enriched diet, $p<0.01$. Incidence of severe osteoarthritis. Lard-enriched diet: cottonseed oil-enriched diet, $p<0.001$.

30% that of osteoarthritis increased. Maximal arthritic involvement of 70% was observed at a mean age of 25.2 months. Irrespective of age at death 26 of a total of 55 mice fed the vegetable fat (47%) had osteoarthritis at a mean age of 20.1 months. Of the 26 arthritic joints 11 (42%) showed severe lesions.

The following Table 2 represents a condensed summary of the articular findings obtained in mice of Group C and those of Groups A and B respectively.

The respective mean ages at death of mice of Groups A and C were 20.1 and 20.3 months. The total incidences of joint disease were 43% and 47% respectively and thus were similar for both groups. However, there occurred a slight shift in the peak incidence. Animals of Group A showed maximal involvement of joints (96%) at the mean age of 22.2 months, after which age there was a decrease of joint disease.[1] Mice of Group C by contrast showed a high of 70% at a mean age of 25.2 months. However, this difference is not significant statistically. Of the joint lesions 55% were severe in stock-fed mice and 43% in the mice kept on the diet enriched with cottonseed oil. Thus as compared to stock-fed controls neither the severity nor the incidence of the joint lesions were significantly altered by consumption of the diet enriched with cottonseed oil.

The observations made in the joints of mice fed the vegetable fat thus differed distinctly from those established for mice given a similar quantity of lard as a dietary supplement. As stated previously,[1] the harmful effect of lard manifested itself in an accelerated onset and an increased incidence and accentuated severity of the articular lesions. Among a total of 78 lard-fed mice 57 (73%) had osteoarthritis as compared to an incidence of 47% found in animals that had received cottonseed oil as a dietary supplement. This difference is statistically significant at the 1% level, and accentuated by the fact that animals receiving cottonseed oil lived on the average two months longer than those given lard. Moreover, only 42% of the joint lesions were severe after consumption of cottonseed oil as compared to 86% after intake of lard. This difference is likewise highly significant ($P<0.001$).

Comment

In male mice of strain C57BL, a stock ration enriched with 25% cottonseed oil did not adversely affect the course of articular aging and of osteoarthritis as had been observed in animals fed the stock ration enriched with 25% lard.[1,2]

The mechanism by which lard acts on articular cartilage is not adequately understood. However, the failure of cottonseed oil to act similarly may give a lead for further investigations into the nature of the lard effect. The local effects of fats are possibly related to their respective action on the serum level of lipids. In humans consuming homogenous or mixed diets, animal fat increased and vegetable fat decreased the plasma lipid concentrations.[6,8] Further-

more, in the fat depots of rats fed iodine-labeled cottonseed oil 70% more fat was deposited than was in the depots of rats fed iodine-labeled lard.[8] Moreover, there seem to be differences in the specific effects of various vegetable fats on the mobility and storage of cholesterol. Thus in rats, the increase of plasma lipids and the storage of cholesterol esters in the liver were higher after consumption of cottonseed oil than after consumption of coconut oil.[7] Vegetable fats may thus contain a substance or substances that influence the sterol and phospholipid levels of the serum.

In this connection the divergent action of lard and cottonseed oil on life span is of interest. While in mice of strain C57BL lard shortened the life span,[1,4] an adverse effect of cottonseed oil on the length of life could not be established. Similar differences in the effects of fats of various origin exist also in regard to the life span of rats.[9]

More and more evidence accumulates showing that the biological actions of fats may be partly determined by their contents of saturated or unsaturated fatty acids respectively as well as by the length of their chains.[5,8] It is therefore not impossible that the failure of cottonseed oil to injure the articular cartilage may also be—directly or indirectly—related to the chemical composition of this fat. Conditions would then be comparable to the respective roles of animal and vegetable fat in the induction of atherosclerosis. Whether or not these observations apply to the articular effects of animal fats and vegetable fats in general requires further study.

A link between the effects of fats on serum chemistry on the one hand and on articular aging on the other may be furnished by observations regarding the action of various fats on skeletal growth.[3,10,11] Agents that stimulate growth accelerate articular aging and intensify the course of degenerative joint disease.[1,2] Since animal fats exert a more marked growth stimulation than vegetable fats,[10] lard might be expected to promote articular aging and osteoarthritis more conspicuously than cottonseed oil does. The failure of the latter to alter the development of osteoarthritis would thus be in keeping with its systemic effects. The present findings again demonstrate a parallelism of articular aging and the arthropathy of old age, and thus provide additional support for the view that the two processes are correlated phenomena.[1,12]

Summary

In male mice of strain C57BL, a ration containing 25% cottonseed oil did not adversely affect the skeletal time curve, the evolution of osteoarthritis or the length of life. A dietary supplement of cottonseed oil was thus less injurious than one of lard. This finding is thought to be related to differences in the systemic actions of lard and cottonseed oil respectively, in particular to differences in their effects on skeletal growth.

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Modifying Effect of Linoleic Acid on Articular Aging and Osteoarthrosis in Lard-Fed Mice*

By M. Silberberg, Ruth Silberberg and Betty Orcutt

In male mice of strain C57BL consumption of a diet enriched with lard from the time of weaning through life increased the incidence, accentuated the severity and accelerated the onset of articular aging changes and of senile osteoarthrosis[8]. Conversely, a diet enriched with cottonseed oil failed to modify materially the development of joint disease of mice of the same strain[9]. This difference in the response to dietary fats was tentatively attributed to variations in the composition of the fats, in particular to the presence of unsaturated fatty acids in the vegetable oil or their absence in lard respectively[9]. In order to test this assumption, it was thought of interest to study articular aging and osteoarthrosis in old mice fed a lard-enriched diet supplemented with one of the unsaturated fatty acids.

Material and Methods

Ninety-eight male mice of the inbred strain C57BL 1x6 were used.

Group I. Thirty mice were kept on a stock diet of Purina laboratory chow. This ration contains 5% animal fat and its caloric value is 4.2 cal/g.

Group II. Thirty-three mice were from the age of one month fed a diet enriched with 25% lard**. This ration was prepared by grinding the laboratory chow to a fine

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** Swifts "Silverleaf" brand.

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meal, mixing it thoroughly with the lard and adding 5% of Fortified Vitamin Mix*. The composition of this ration was:

Purina laboratory chow	1155 g
Lard	435 g
Fortified Vitamin Mix	75 g

This diet has a caloric value of 5.4 cal/g.

Group III. Thirty-five mice received from the age of one month a diet enriched with 23% lard and 3% unsaturated fatty acid. This ration which has also a caloric value of 5.4 cal/g was composed as follows:

Purina laboratory chow	1155 g
Lard	385 g
Linoleic acid	50 g
Fortified Vitamin Mix	75 g

The animals were fed ad libitum with water available at all times.

The food intake was determined daily up to the age of 4 months, and thereafter during one week of each month. The mice were killed at the age of 18 months except for those that died or had to be killed prematurely.

Complete necropsies were done. Tibias and femurs including the kneejoint were removed as a whole, fixed and decalcified in Bouin's solution and embedded in paraffin. Serial sections were stained with hematoxylin and eosin. The results of the microscopic examination were evaluated statistically by the χ^2 test using Yates' correction for continuity.

Observations

Gross. On the whole, the experimental diets were well tolerated, and no diseases were observed during the experiment. Four animals of each experimental group suffered marked loss of weight, died or had to be killed prematurely; post mortem examination failed to disclose anatomical causes of death.

Weight. The animals fed the lard-enriched diet showed the fastest and most marked weight increases of the three groups. Maximum weights (mean 36.4 g) were present at the age of 12 months. At 15 months of age a slight drop, and thereafter a steep fall to a mean weight of 26.7 g occurred. This was the lowest mean weight seen in any of the three groups. The mice fed linoleic acid had their maximum weights from 12 to 15 months of age (mean: 33.4 g). Subsequently the mean weights dropped to 31.0 g. The stock-fed controls did not reach the maximum weights until the age of 15 months, the mean (32.3 g) remaining below those of the other groups, and had not yet begun to lose weight at the age of 18 months. Individual variations were by far less marked than in mice fed the fat-enriched diets.

* Obtained from Nutritional Biochemical Co., Cleveland, Ohio.

Table I

Showing Mean Weights and Their Ranges of Stock-Fed and Test Animals

Diet	No. of mice	6 months	12 months	15 months	18 months
Stock	30	26.6 (25–28)	31.1 (29–31)	32.3 (28–34)	32.2 (27–33)
Lard	33	29.8 (28–34)	36.4 (31–49)	35.2 (31–47)	26.7 (20–34)
Lard + Linoleic acid	35	28.5 (26–32)	33.3 (20–45)	33.4 (17–46)	31.0 (18–44)

Food intake. The mean total intake and the caloric intake per gram body weight at the ages of 1, 6, 12, and 16 months are summarizes in Table II.

Table II

Showing the Average Total Caloric Intake and the cal/g Intake in Stock-Fed and Test Animals at the Ages of 1, 6, 12, and 16 Months

Diet	No. of mice	At 1 month Tot. cal.	cal/g	At 6 months Tot. cal.	cal/g	At 12 months Tot. cal.	cal/g	At 16 months Tot. cal.	cal/g
Stock	30	9.1	1.0	11.2	0.7	12.1	0.5	11.9	0.4
Lard	33	12.4	0.7	14.6	0.5	14.6	0.4	18.4	0.5
Lard + Linoleic Acid	35	14.9	1.0	14.9	0.5	17.4	0.5	16.4	0.5

In terms of total caloric intake the mice consumed more of the enriched rations than those on the stock diet, the difference reaching about 20% at 6 months and rising to about 50% at 16 months of age. Within each group, the caloric intake per gram body weight during the growth period was consistently higher (1.0 or 0.7 cal) than after cessation of growth (0.7–0.4 cal/g). From the age of 12 months on, the caloric intake per gram body weight was relatively uniform (0.4–0.5 cal/g) regardless of the diet consumed.

Microscopic findings. With advancing age, the chondrocytes may undergo hyperplasia and hypertrophy, while the articular surface remains intact. In advanced stages, regressive changes begin to predominate with irregularities, roughening or slight fraying of the surface. A combination of growth and regressive changes gives rise to osteoarthrosis of hypertrophic, ulcerative or mixed type (Fig. 1,2).

The findings in the kneejoints of stock-fed mice and of mice fed the experimental diets are summarized in Table III, which shows

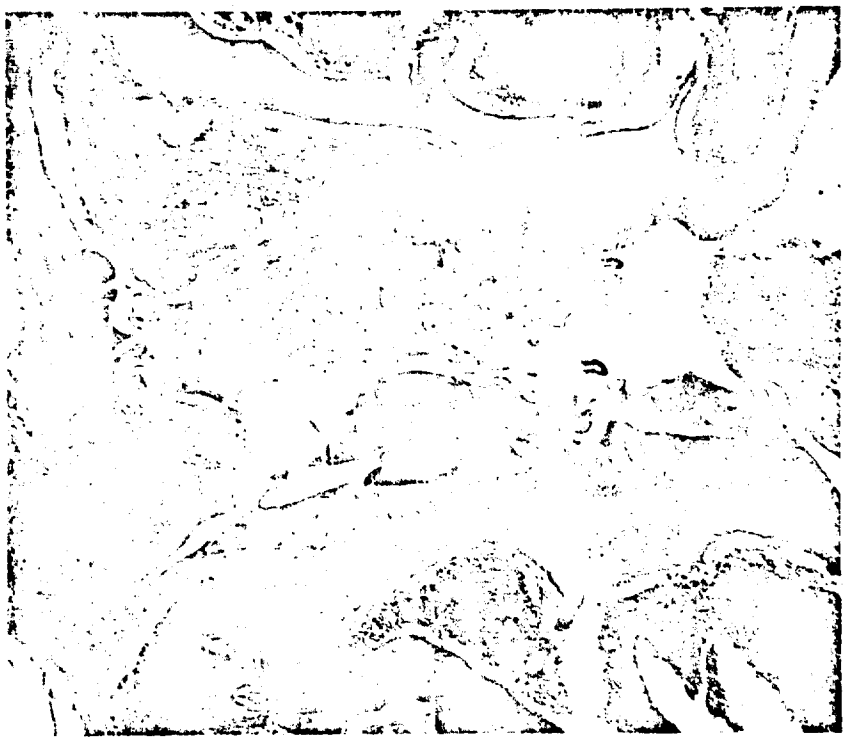

Fig. 1. Osteoarthrotic kneejoint of a mouse. Femur in upper, tibia in lower half of picture. Proliferation of cartilage at meniscus defect, at femur and about defect at tibial surface. Proliferation and thickening of synovialis and beginning fibrosis of tibial epiphysis. x 70.

the incidences of unchanged joints, of minor or advanced aging changes and of osteoarthrosis.

Of animals fed the stock diet 20% had unaltered joints, 53%

Table III

Showing the Articular Findings in Stock-Fed and Test Animals

Diet	No. of mice	Mean age at death months	No change No.	No change %	Aging changes slight No.	Aging changes slight %	Aging changes advanced No.	Aging changes advanced %	Osteoarthrosis No.	Osteoarthrosis %
Stock	30	18.0	6	20	7	23	9	30	8	27
Lard	33	17.8	0	0	2	6	6	18	25	76
Lard + linoleic acid	35	17.7	0	0	10	29	11	31	14	40

Significance of differences: Lard: Stock diet $p < 0.001$. Lard: Lard + linoleic acid $p \quad 0.01$.

Fig. 2. Lateral part of osteoarthrotic kneejoint of a mouse. Ossifying cartilaginous marginal outgrowth at tibia. x 70.

had aging changes of varying severity, and 27 % had osteoarthrosis. There were no unchanged kneejoints among the animals fed the enriched diets. Of the animals fed the lard-enriched diet 24 % had slight or severe aging changes, while in 76 % osteoarthrosis was found. This increase over the spontaneous incidence of osteoarthrosis in untreated controls is highly significant ($p < 0.001$). Mice consuming the lard-enriched ration containing linoleic acid disclosed about the same incidence of aging changes as the untreated controls. However, the difference in the incidence of osteoarthrosis in mice receiving lard only and those fed the combination of lard and linoleic acid respectively (40 %) was significant at the 1 % level.

Discussion

A lard-enriched diet given to male mice of strain C57BL from the time of weaning to the age of 18 months accelerated the onset of aging changes and significantly increased the incidence of osteoarthrosis as compared to that seen in stock-fed mice. These results are in agreement with those of previous experiments in which lard was fed to mice[8] and Crisco to rats[11]. Substitution of part of the lard by linoleic acid at a 3% level resulted in a statistically significant lowering ($p < 0.01$) of the incidence of osteoarthrosis as compared to that noted in animals fed lard without the supplement of linoleic acid. The relative increase of aging changes in association with the decrease in the number of arthrotic joints after consumption of the linoleic acid diet suggests a retardation of the processes resulting in joint disease as compared to the course of events in mice fed a lard-enriched diet without added linoleic acid. The facts that, unlike in stock-fed mice, unaltered joints were absent after ingestion of the linoleic acid ration and that osteoarthrosis was still more common than in stock-fed controls indicate that the injurious effect of the lard diet was mitigated but not abolished by the addition of linoleic acid to the ration. Whether or not it is possible to prevent the harmful effect of the lard-enriched diet altogether by altering the concentration or the type of unsaturated fatty acid will have to be determined by further experiments. However, even if this could be accomplished, it would not permit the conclusion that linoleic acid prevents the development of spontaneous osteoarthrosis under all conditions.

Strain differences exist not only in the incidence of spontaneous osteoarthrosis but also in regard to the arthrosis - promoting effect of a lard-enriched diet - the joints of DBA males being hardly affected by such a diet[10], an observation recently confirmed on still another subline of this strain[12]. Although due to genetic mutation the susceptibility of individual strains to spontaneous and to induced arthrosis may change over the years, that of males of four subline of strain C57 has remained fairly constant. Moreover, in all investigations of senile osteoarthrosis the role of age must not be overlooked; especially negative results obtained in middle-aged animals cannot be extrapolated to apply to conditions present at older ages.

The results obtained correlate with the attenuation of other harmful effects of saturated fats with short chains or of cholesterol

by some unsaturated fatty acids, such as the development of atherosclerosis and fatty livers in diabetic and non-diabetic patients[2,3], in rabbits[4,11], rats[1,2] and pigeons[6]. According to some of these studies the attenuation of vascular and hepatic lesions induced by diets high in saturated fat was associated with a lowering of serum cholesterol or a decrease of hyperlipemia[3,11] as well as of hepatic sterolesters and triglycerides[7]. However, there were differences in the mode of action of various unsaturated fatty acids depending on their chemical structure and on the dose given[2,5]. In pigs, a dietary supplement of less than 1% linoleic acid had a favorable effect on lipemia induced by olive oil, while higher or lower doses of linoleic acid were harmful or ineffective[5]. In experimentally produced aortic lesions and fatty livers, the fatty acid components of cholesterol esters varied with the fatty acids used[13].

The mechanism by which unsaturated fatty acids modify the harmful effects of saturated fatty acids is still being discussed in relation to extraskeletal tissues. As regards the articular cartilage the point of attack of the injury imparted by saturated fat is still unknown, let alone the nature of the attenuating action of unsaturated fatty acids. The latter may either be involved in a central phase of lipid metabolism or in a phase of lipid metabolism common to a variety of cells including articular chondrocytes.

As far as the relationship between body weight and joint disease is concerned, the present results confirm our previous observations that overweight as such may play a role in the pathogenesis of osteoarthrosis only, if it reaches extraordinary values. In group II, 7 mice weighed more than 45 g – that is about 50% above the mean maximum of stock-fed controls –; 6 of these mice had osteoarthrosis. In group III, two mice had reached this high weight, but neither of them had developed joint disease.

Acknowledgment. We are indebted to Dr. *E. Wyler*, Uni-Chemie, Zurich (Switzerland) for a generous supply of linoleic acid.

Summary

In the kneejoints of male mice of strain C57BL Jax6, a dietary supplement of 3% linoleic acid partly reversed the injurious effect caused by a lard-enriched ration not containing unsaturated fatty acids. The incidence of osteoarthrosis which was almost tripled by consumption of the lard-enriched diet, was only insignificantly

increased above the spontaneous level in mice given the linoleic acid supplement. There was no correlation between the gain in body weight and the incidence of joint disease. However, while the mice fed the lard-enriched diet suffered considerable weight loss in middle age, those consuming the linoleic acid diet held their weights at near normal levels. It is concluded that the beneficial effect of linoleic acid on the injurious action exerted by a lard-enriched diet on the articular tissues is basically comparable to corresponding observations in non-skeletal tissues.

Zusammenfassung

Zusatz von 3% Linolensäure zu einer Schmalzdiät setzte bei männlichen Mäusen des Stammes C57BL Jax6 die schädigende Wirkung auf die Kniegelenke herab, die ohne Beigabe von ungesättigten Fettsäuren beobachtet wurde. Die Häufigkeit der Altersarthrose, die unter dem Einfluß der Schmalzdiät den Kontrollmäusen gegenüber nahezu verdreifacht war, wurde durch Linolensäurezusatz so weit herabgesetzt, daß sie nur unwesentlich von derjenigen abwich, die man nach Verfütterung der Standarddiät findet. Eine Beziehung zwischen Gewichtszunahme und Gelenkerkrankung bei den fett-gefütterten Tieren konnte nicht festgestellt werden. Tiere, die die Schmalzdiät erhielten, zeigten in mittlerem Lebensalter erheblichen Gewichtsverlust im Gegensatz zu denen, die zusätzlich mit Linolensäure gefüttert wurden. Es wird gefolgert, daß die günstige Wirkung der Linolensäure auf die Schädigung der Gelenke durch die Schmalzernährung grundsätzlich ähnlichen Beobachtungen an anderen Geweben entspricht.

Résumé

L'administration de l'acide linolénique à 3% diminue l'action nocive du régime riche en lard et pauvre en acides gras non saturés sur les articulations du genou chez la souris de souche C57BL Jax6. En plus, l'arthrose sénile, qui — par rapport à la normale — est trois fois plus élevée chez les souris nourries au lard, est réduite à un chiffre à peu près normal. Il n'a pas été observé de relation entre le degré de l'obésité et l'importance des lésions articulaires chez les souris nourries au lard. L'importante perte de poids, observée chez les souris mangeant du lard à l'âge moyen est empêchée par l'administration supplémentaire d'acide linolénique. Il est conclu que l'acide linolénique exerce la même action favorable sur les lésions articulaires que sur les autres lésions tissulaires provoquées par le régime riche en lard.

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EFFECTS OF A HIGH FAT DIET ON THE JOINTS OF AGING MICE

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ST. LOUIS

IN YOUNG MICE of strain C57 black reared on a diet containing 30 per cent fat, skeletal growth and development were accelerated. The hastening of growth processes was, however, associated with, or followed by, premature skeletal aging. The changes noted in the articular tissues were interpreted as early manifestations of degenerative joint disease.[1] The present report deals with observations extending over the life span of the mouse. The articular changes taking place under the influence of the high fat diet were studied and compared with those occurring spontaneously in mice receiving a stock diet containing 5 per cent fat.

MATERIAL AND METHODS

Two hundred and fifty male mice of the closely inbred strain C57 black, raised in our laboratory, were divided into two groups, littermates being equally distributed between the two groups whenever possible. The animals of the first group (control animals) were fed a commercial chow.[2] The animals of the second group (test animals) were, from the time of weaning, kept on a fat enriched diet composed of the stock ration ground to a meal, with 25 per cent lard[3] added. Both diets were given ad libitum, with water available at all times.

From the Snodgras Laboratory of Pathology, Hospital Division, City of St. Louis, and the Department of Pathology, Washington University School of Medicine.

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2. This was the purina® laboratory chow known as ration B 2362, made by the Ralston Purina Company, St. Louis.

3. The silverleaf brand, marketed by Swift & Company, was used.

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The composition of the control and experimental diets was, as follows:

	Control Diet	Experimental Diet
Moisture, per cent	8.90	6.7
Protein, per cent	26.18	19.6
Fat, per cent	5.35	29.0
Fiber, per cent	4.62	3.5
Ash, per cent	6.49	4.9
Nitrogen-free extract, per cent	48.46	36.3
Calcium, per cent	1.17	0.9
Phosphorus, per cent	0.97	0.7
Magnesium, per cent	0.196	0.147
Iron, parts per million	327.0	245.0
Manganese, parts per million	106.0	88.0
Copper, parts per million	16.8	12.6
Cobalt, parts per million	0.14	0.11
Potassium, per cent	0.90	0.68
Carotene, parts per million	6.6	5.0
Thiamine, parts per million	12.85	9.7
Riboflavin, parts per million	7.49	5.6
Niacin, parts per million	65.6	49.2
Vitamin D, U. S. P., units per Gm	4.97	3.75
Vitamin A, U. S. P., units per Gm	9.0	6.75

The experimental diet was thus adequate in all respects.[4] The protein content, 19.6 per cent, is slightly below the minimum requirement, 20 per cent. However, this deficiency of 0.4 per cent should be amply made up by the increased utilization of protein observed in the presence in the ration of large amounts of fat.[5]

The original plan was to sacrifice groups of 25 animals each at 9, 12, 15, 18 and 24 months of age. However, some animals died prematurely or had to be killed because they appeared ill. In addition, with the high fat diet it was difficult to keep mice alive beyond the age of 18 months. Between the ages of 15 and 18 months there often was a sudden loss of weight which continued for some time, so that the animals had to be killed at irregular intervals. This circumstance accounts for the comparatively large number of test animals in the group 13 to 18 months of age. Some animals which had died and whose tissues had undergone autolysis were eliminated. Altogether, the bones of 113 mice fed the regular diet and of 111 mice fed the high fat diet were available for microscopic examination (table 2).

At necropsy, the individual weight was taken, and pathological findings were recorded. Both legs were removed as a whole, and diseased internal organs, as well as pieces of grossly unchanged tissues, were saved. The legs were freed from the soft tissues, and fixed and decalcified in Bouin's solution. Decalcification was accomplished within six to eight days with one change of fluid after three or four days. The bones were then split by a sagittal cut; one half of a leg was dehydrated in the usual manner and embedded in paraffin; another half to be stained for glycogen was dehydrated in dioxane and embedded in paraffin; still another half was kept in 4 per cent formaldehyde solution for other special stains—in particular, fat stains. As a routine, semiserial sections were stained with hematoxylin and eosin.

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GROSS OBSERVATIONS

Weights.—Throughout the second year of life the mean weights of the control animals were, on the whole, constant, varying from 31 to 33 Gm.; from the end of the second year of life on, the weights declined steadily. The weight curve of the test animals roughly paralleled that of the controls, disclosing an over-all increase of about 5 Gm. (15 per cent) over the controls.

The peak seen in the curve of the test animals requires some comment: At the age when this high occurred, the majority of the mice fed the high fat diet were dead; the survivors were either animals with exceptionally high weights or animals in which the terminal loss of

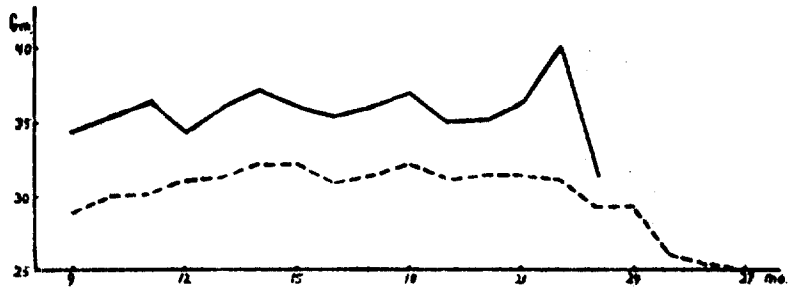

Fig. 1.—The mean weights of control and test mice, respectively. The broken line represents the mean weight curve of control animals; the solid line, that of test animals.

TABLE 1.—*Monthly Mean Weights of the Control and Test Animals, with Maximum and Minimum Deviations*

Age, Mo.	Control Animals			Test Animals		
	Mean, Gm.	Deviation, Gm.		Mean, Gm.	Deviation, Gm.	
		Maximum	Minimum		Maximum	Minimum
9	29.0	4.0	2.0	34.6	5.4	3.6
10	30.1	2.9	3.1	35.4	10.6	2.4
11	30.2	2.8	1.2	36.3	10.7	4.3
12	31.1	3.9	2.1	34.3	13.7	4.3
13	31.3	3.7	6.3	36.0	13.0	6.0
14	32.2	3.8	1.2	37.0	22.0	8.0
15	32.3	6.7	5.3	36.1	28.9	5.1
16	31.0	5.0	3.0	35.4	12.6	6.4
17	31.4	3.6	3.4	36.0	18.0	8.0
18	32.2	2.8	4.2	36.8	22.2	9.8
19	31.1	3.9	3.1	35.0	10.0	8.0
20	31.4	3.6	3.4	35.1	12.9	8.1
21	31.4	3.6	3.4	36.2	14.8	4.2
22	31.1	3.9	2.1	40.1	4.9	5.1
23	29.4	1.6	3.4	31.1	0.9	1.1
24	29.3	2.7	2.3	....	....	...
25	26.0	1.0	1.0	....	....	...
26	25.4	0.6	0.4	....	....	...
27	25.0	2.0	1.0	....	....	...
28	26.0	0.5	0.5	....	....	...
29	25.0	1.0	1.0	....	....	...
30	25.0	...	...	....	....	...

weight had not yet taken place. If this small group of selected animals had been excluded, this peak would not be noticeable, and the curve would show instead a gradual decline from the age of 15 months on.

Moreover, the two curves showing the mean weights give only an incomplete picture of the actual conditions without supplementary data on the weight ranges in the control and the test animals, respectively. These ranges appear in table 1 and are graphically demonstrated in figure 2 as deviations from the mean weights.

In figure 2 the area bordered by the two solid lines shows the weight ranges found in the test animals; the area bordered by the broken lines,

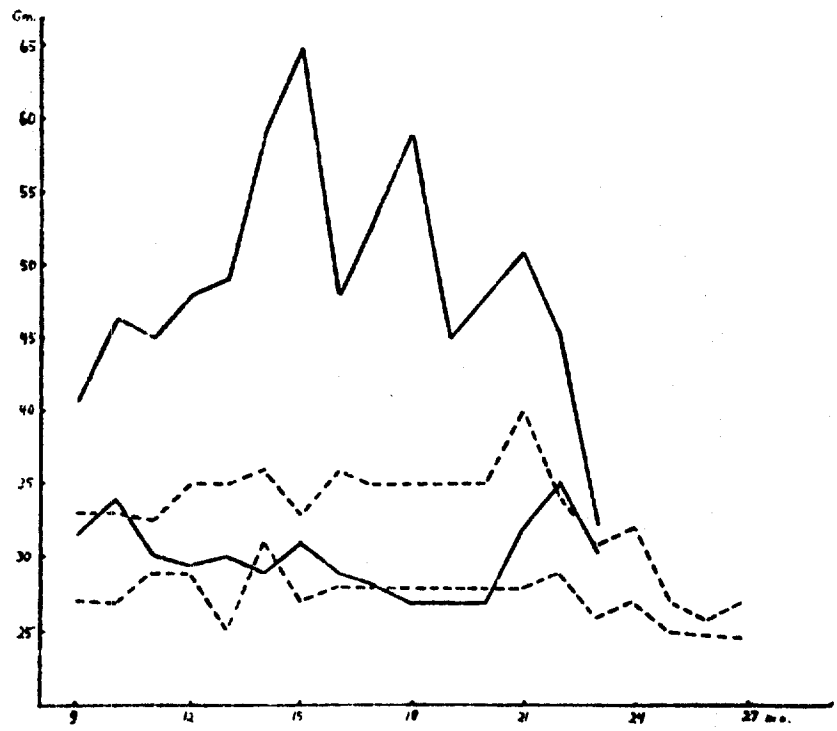

Fig. 2.—The ranges of the weights of control and test mice, respectively. The two broken lines represent the maximum and the minimum weight curves of the controls; the two solid lines, the maximum and minimum weight curves of the test mice.

the weight ranges observed in the controls. In the control group the variations in weight were by far more limited than in the test group. The highest weight ever observed in the control series was 40 Gm.; that is 5 Gm., or about 10 per cent, above the mean found in this group. In the test series the highest weight ever observed was 65 Gm.; that is 28.9 Gm., or about 85 per cent, above the mean in this group. Particularly among the test animals wide variations in weight were noticeable, even within a group of littermates kept in the same cage.

Findings at Necropsy.—In those test animals in which considerable overweight had developed, visceromegaly and extensive deposits of adipose tissue were found in the subcutaneous tissue and in the abdominal cavity. Contact roentgenograms disclosed slightly increased density of the bones.

MICROSCOPIC OBSERVATIONS

The criteria used in the following classification of the articular changes are similar to those established previously.[6a]

No Change.—Starting from the articular surface and proceeding toward the epiphysis three cell layers may be distinguished: the sliding zone, containing one to three rows of undifferentiated spindle-shaped cells, the axes of which are are oriented parallel to the surface of the joint; the transitional zone, containing small ovoid cells separated from each other by abundant amounts of homogeneous chondromucoid ground substance; the zone of ossifying cartilage. An osseous lamella of varying thickness delimits the cartilaginous covering from the epiphysial marrow (fig. 3 *A*).

The cells of the articular surface thus undergo a cycle of growth, development and senescence similar to that of cells of the epiphysial growth zones. However, whereas in the latter this cycle ceases at the end of the growth period, in the joints it continues throughout life, though at a diminishing and ultimately extremely slow rate.[6b,c] The ligaments are composed of collagenous stroma containing a few fibrocytes and, near the insertion at the articular surface, some precartilage cells. The menisci consist of fibrillar connective tissue, fibrocartilage or hyaline cartilage; the membrana synovialis is composed of loose vascular connective tissue covered by a continuous single layer of mesothelial cells.

Age Changes.—Slight Changes: The articular cartilage reveals growth processes involving the entire articular surface more or less uniformly. The undifferentiated cells of the surface layer round off, begin to enlarge, proliferate and become more closely packed than ordinarily; mitoses may be found. The hyperplastic processes are particularly striking in the intermediate zone (figs. 3 *B* and *C*). Occasionally enlarged cartilage cells are surrounded by a halo of basophilic matrix. More often, however, growth processes occur without demonstrable changes in the matrix, at least in sections stained with hematoxylin and eosin.

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6. Silberberg, M., and Silberberg, R.: (*a*) Am. J. Anat. **68**:69-95, 1941; (*b*) The Effects of Endocrines on Articular Tissues and Their Relation to Ageing Processes, in Proceedings of the Seventh International Congress on Rheumatic Diseases, 1949, Philadelphia, W. B. Saunders Company, to be published; (*c*) Growth **13**:359-368, 1949.

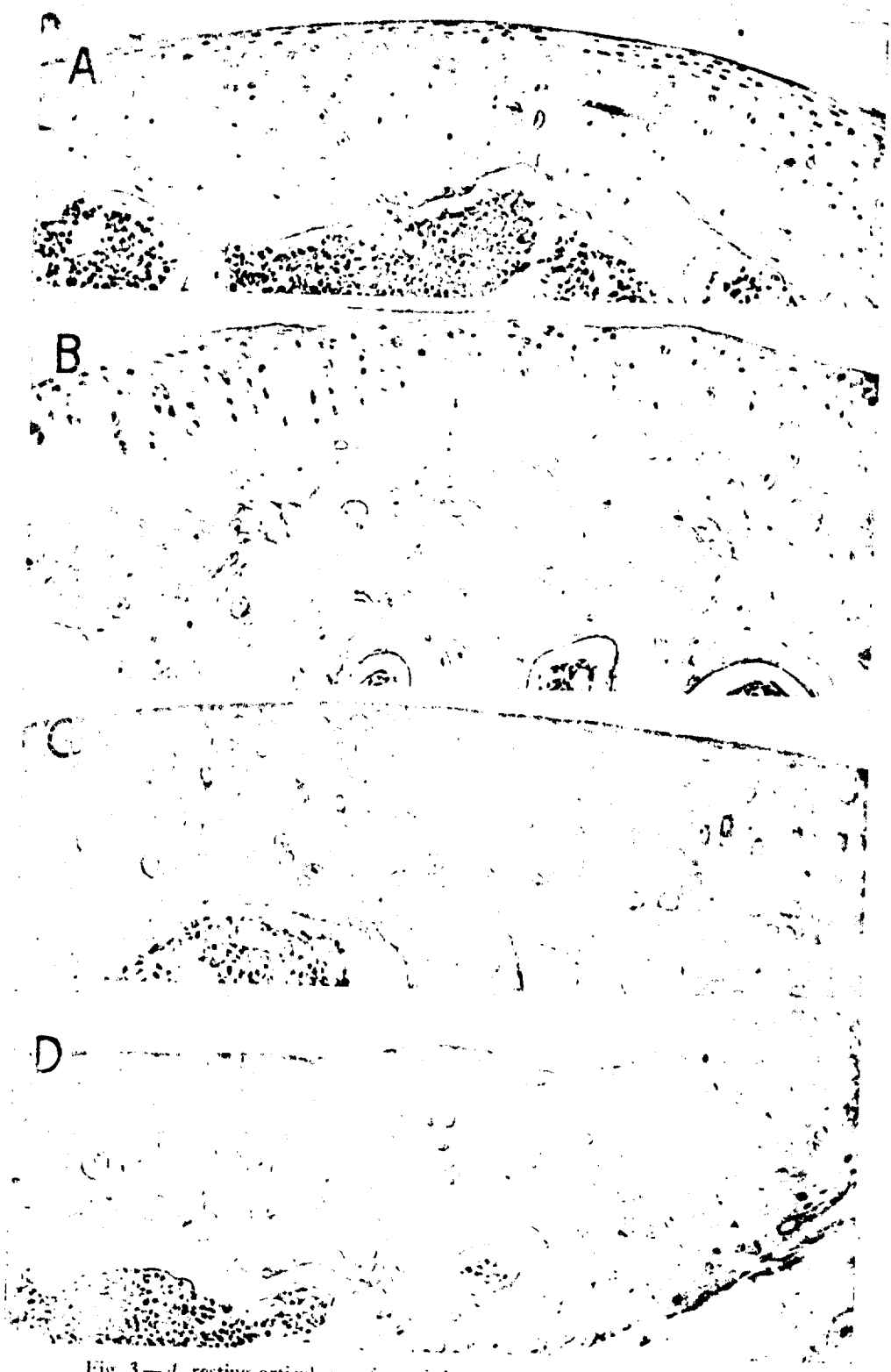

Fig. 3.—*A*, resting articular surface of the upper end of the tibia; 22 month old control mouse; × 200.

B, slight hyperplasia and hypertrophy of the articular cartilage at the upper end of the tibia; 15 month old control mouse; × 200.

C, moderate hyperplasia and hypertrophy of the articular cartilage at the upper end of the tibia; 12 month old control mouse; × 200.

D, hyperplasia, hypertrophy and marked regressive change in cartilage cells and matrix at the surface of the patella; 9 month old test mouse; × 200.

Increasingly from the age of 12 months on, the ligaments undergo cartilagination; the menisci begin to have centers of marrow and ossify. Moreover, the membrana synovialis may proliferate (fig. 4) and show swelling or fibrinoid change. Proliferation usually sets in near the insertion of the cruciate ligaments, and from here the areolar tissue may spread into the epiphysial marrow through dilated vascular channels. Thus foci of fibrosis of varying density appear in the epiphysis (fig. 5 *A* and *B*). The changes in the synovialis are not associated with inflammatory processes, and they may occur without changes in the cartilage.

Fig. 4.—Thickened and proliferating membrana synovialis; 22 month old control mouse; × 200.

Advanced Changes: The articular cartilage reveals intensification of growth processes and prominence of regressive changes (fig. 3 *D*). Hypertrophic cartilage cells with pyknotic nuclei and basophilic halos appear in increasing numbers. Unlike the cells of the growing joint which undergo the regular cycle of development, these enlarged cells remain near the surface; furthermore, they do not show the vacuolation typical of differentiating cartilage cells. Focal proliferation of cartilage cells may be observed, leading to the appearance of small clusters adjacent to areas of degeneration. These changes go hand in hand with marked basophilia of the matrix and focal irregularities such as fraying or roughening of the surface, especially near the insertion of the liga-

ments. If these changes are accompanied by alterations in the ligaments or the synovialis, they merge gradually into the picture of osteoarthritis.

Osteoarthritis.—The prominent features are severe distortions of the usual structure of the joint (figs. 5 *C* to 9). The surfaces of femur, tibia and patella may be affected. The changes vary in kind and show all com-

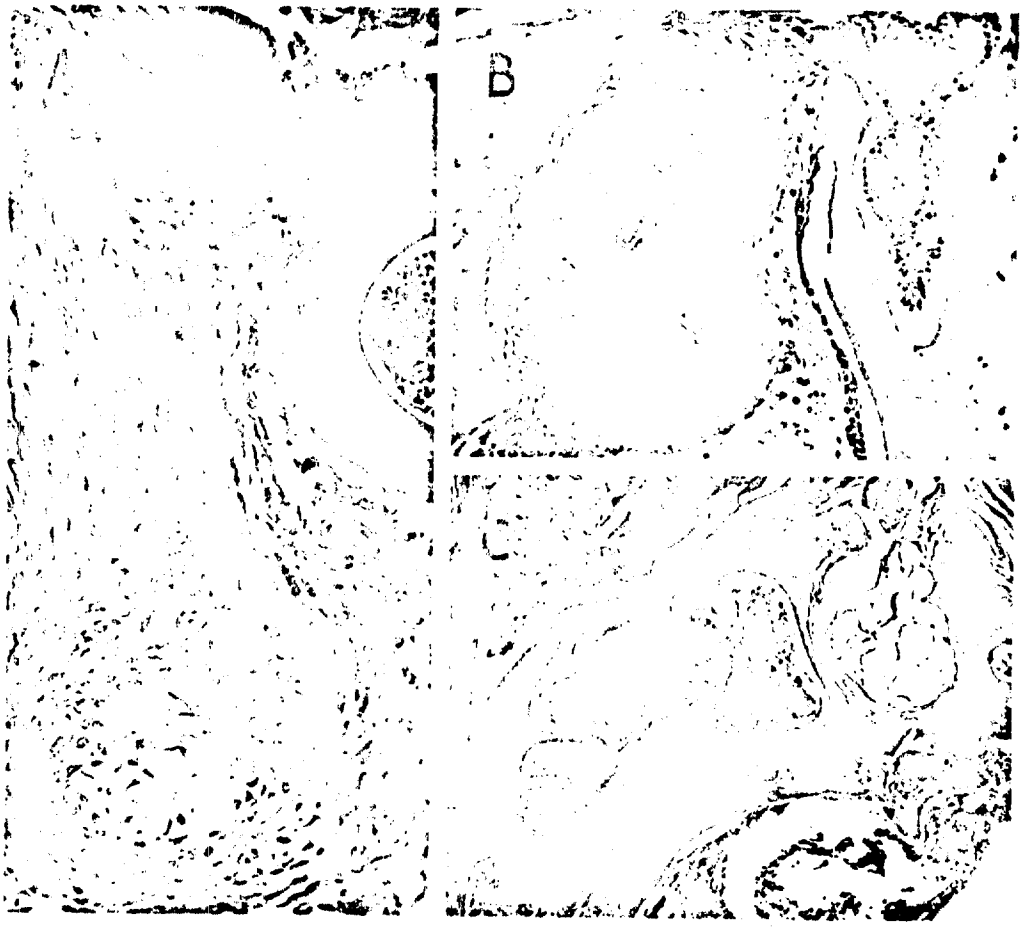

Fig. 5.—*A*, fibrosis and swelling of the membrane synovialis, with connective tissue beginning to grow into the epiphysis of the tibia through the bone of the surface of the joint; 18 month old control mouse; × 200.

B, fibrous replacement of the marrow of the epiphysis of the tibia, representing a stage which is more advanced than that shown in *A*; 18 month old test mouse; × 200.

C, fibrosis and cyst formation in the marrow of the epiphysis of the tibia; the persisting epiphysial plate is visible in the lower part of the photograph; 18 month old test mouse; × 45.

binations of growth processes and regressive changes. The former are hypertrophy and proliferation of the articular cartilage, indicated by the

Fig. 6. *A*, hyperplastic foci in the articular cartilage of the tibia; 18 month old control mouse; × 200.

B, cartilaginous overgrowth at the insertion of a ligament of the tibia; 20 month old test mouse; × 200.

C, bony spur at the articular margin of the tibia arising from ossification of the capsule of the joint; 18 month old test mouse; × 200. Skeletal muscle may be seen at the right; the epiphysial plate, at the lower left of the photograph.

Fig. 7.—*A*, osteoarthritic ulcers at the opposing surfaces of tibia and femur; 18 month old test mouse; × 45. There is condensation of the bone at the floor of the ulcers.

B, part of the ulcer of the tibia shown in *A*; × 200. There is eburnation at the floor of the ulcer and hyperplasia of the cartilage at the margin.

occurrence of incubator capsules and considerable overgrowths. At the same time, cartilage cells are destroyed, and the matrix may be loosened and discloses pronounced pericellular basophilia. Foci of liquefaction, grooves and erosions of the articular surface are common. Loss of cartilage in its entire thickness may cause an ulcer, the floor of which is composed of distinctly thickened sclerotic bone (eburnation). This type of lesion often involves both femur and tibia. Owing to the grinding action of the opposing surfaces, the latter become denuded and thus assume a smooth, polished appearance. The edges of such ulcers usually consist of narrowing tongues of cartilage, and there may be reactive

Fig. 8.—Hypertrophic osteoarthritis with marked deformities and overgrowths at the articular surfaces of tibia and femur; 24 month old control mouse; × 45.

hyperplasia of cartilage with formation of irregular prominent outgrowths. Islands of cartilage are attached to the ligaments, or they appear as free bodies in the articular cavity. There is vascularization of cartilage and bone, and at the articular margins, ossification of ligaments with the formation of osseous projections. The synovialis shows proliferation, increased vascularization and fibrosis with infolding or adherence to the surface of the joint. In the ligaments there are advanced fibrinoid change, cartilagination, ossification and sometimes shrinkage. In the epiphysial marrow, cysts, areas of fibrosis or foci of extravasated blood are noticeable.

Owing to the variety and different combinations of these changes, the microscopic picture of the individual joint varies considerably from that of another. The severe joint lesions of old age are frequently characterized by paucity of cells and predominance of processes of hyalinization affecting areas of the cartilaginous covering as well as the synovialis. The ligaments may be shortened and thickened, and contain foci of

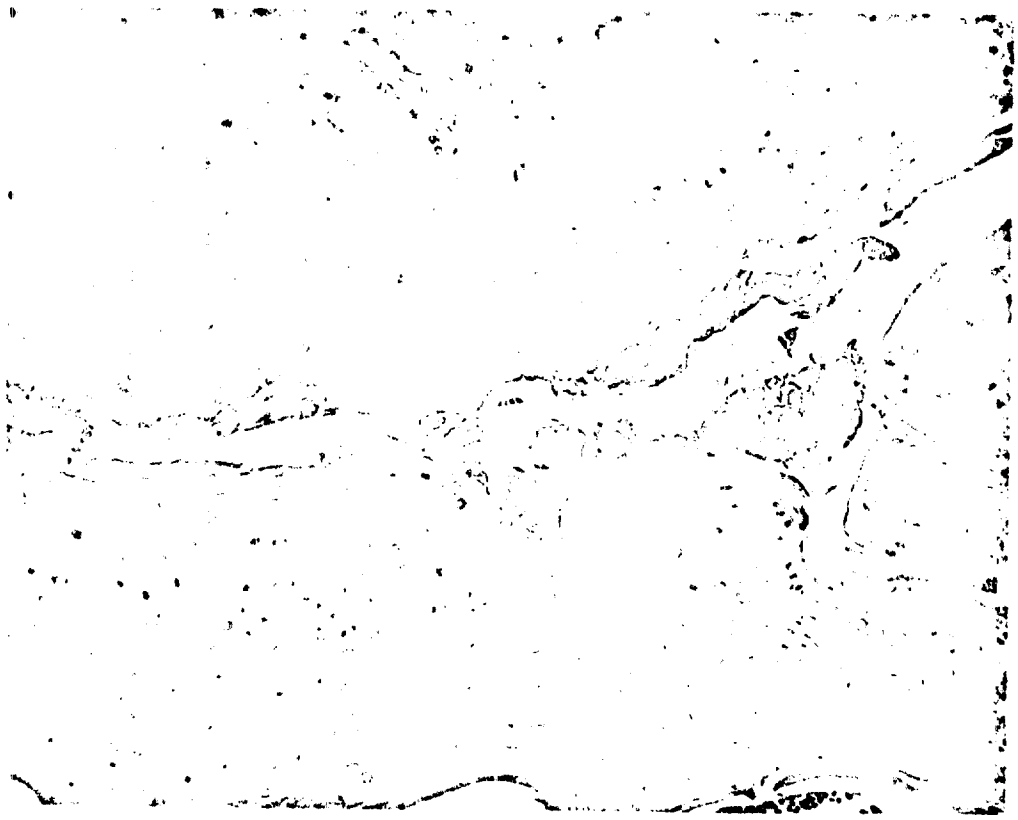

Fig. 9.—Hypertrophic osteoarthritis; 18 month old test mouse; × 200. Marked regressive changes and growth processes are seen at the articular surfaces of the tibia and femur.

hyalinized and ossified cartilage. In these instances one has apparently to deal with "burnt-out" stages of osteoarthritis.

INCIDENCE OF ARTICULAR FINDINGS IN THE VARIOUS AGE GROUPS

The findings in the knee joints are presented in table 2. The animals were divided into the following four age groups: 9 to 12 months; 13 to 18 months; 19 to 24 months; 25 to 30 months. The number of animals

available in each group and their mean ages are shown in vertical columns 3 and 4; the incidence of the findings expressed as number per cent of animals in each age group is given in vertical columns 5 to 8.

At 9 to 12 months of age the knee joints were uninvolved in 22.2 per cent of the controls and in 24.2 per cent of the animals fed the fat-enriched diet. With increasing age the normal joints became fewer in both series of animals. Between 13 and 18 months of age the joints of 11.8 per cent of the controls and of 2 per cent of the test animals were unchanged. Between the ages of 19 and 24 months, 5.6 per cent of the controls had normal joints, and 7.4 per cent of the test animals were free of articular changes, compared with the previous low of 2.0 per cent. Similarly, normal joints were found in 14.3 per cent of the controls living beyond the age of 24 months, compared with the previous low in this group of 5.6 per cent.

TABLE 2.—*The Incidence of Articular Lesions in Various Age Groups*

Age, Mo.	Experimental Group	Mice	Mean Age, Mo.	Percentage of Mice Showing Stated Change: No Change	Age Change: Slight	Age Change: Advanced	Osteoarthritis
9-12	Control	36	10.2	22.2	58.4	11.1	8.3
	Test	33	10.9	24.2	33.3	15.2	27.3
13-18	Control	34	16.8	11.8	49.9	5.9	32.4
	Test	51	16.3	2.0	15.7	13.7	68.6
19-24	Control	36	22.2	5.6	27.7	11.1	55.6
	Test	27	22.0	7.4	0.0	11.1	81.5
25-30	Control	7	27.1	14.3	57.1	0.0	28.6
	Test	0	....	....	....	....	....
9-30 combined	Control	113	17.2	13.3	46.0	8.8	31.9
	Test	111	16.1	9.9	17.1	13.5	59.5

Thus among both the control and the test mice the number of animals with normal joints decreased up to a certain age. However, in the oldest surviving individuals of either group the normal joints were somewhat more frequent than before. In mice fed the high fat diet the decline in the number of unchanged joints was faster and the terminal rise less conspicuous than in the controls. Animals which remain free of articular lesions even in old age may represent a selected group of individuals resistant also to other diseases, and therefore surviving the longest.

In the control group the incidence of slight articular changes decreased from 58.4 per cent at nine months to 27.7 per cent at 24 months; in animals surviving this age a higher incidence (57.1 per cent) was again noted. Owing to the small number of animals available in this group, the rather high incidence of 57.1 per cent is probably not truly representative, but it may well indicate a general trend. Again, these animals may belong to the group of selected individuals resistant to degenerative diseases. The mice, therefore, reach an old age before the articular age changes develop, and during the brief remainder of the life

span no further degeneration of the lesions takes place. Under the influence of the high fat diet the incidence of slight articular changes decreased with advancing age. Between 9 and 12 months of age 33.3 per cent of the test animals showed slight articular changes, compared with 58.4 per cent of the control animals. Between 13 and 18 months of age the incidence dropped rapidly, creating an even more marked difference between control and test animals (49.9 per cent of the controls, 15.7 per cent of the test animals). Slight changes were not recorded in 19 to 24 month old animals fed the high fat diet. The significance of the decrease in slight changes with advancing age in control as well as in test animals can be gaged only in connection with the simultaneous rise of the advanced osteoarthritic lesions. This indicates that the age changes are transformed into the severe types of degenerative joint disease with advancing age as well as under the influence of the high fat diet.

There were only slight variations in the incidence of advanced articular age changes with increasing age or with the change in the diet (table 2, column 7). These findings suggest that the advanced age changes represent a transitional stage from which the articular tissues of susceptible animals pass quickly into the osteoarthritic stage, and which, on the other hand, is not reached by animals resistant to articular disease.

Osteoarthritis was observed in a small number (8.3 per cent) of control animals 9 to 12 months of age. However, the incidence of these lesions increased with advancing age until at the end of the second year of life more than one half (55.6 per cent) of animals were thus affected. Of animals living into the third year of life 28.6 per cent had osteoarthritis. However, this percentage should be evaluated with the fact in mind that only few animals survived to this age. At the end of the first year of life osteoarthritis was three times as frequent in animals fed the high fat diet (27.3 per cent) as in the controls (8.3 per cent). At 18 and 24 months of age the incidence of osteoarthritis had increased in the test animals to 68.6 per cent and 81.5 per cent, respectively, compared with 32.4 per cent and 55.6 per cent in control animals of corresponding age. Thus, as had been found in controls, in the test animals osteoarthritis also became more frequent with advancing age.

In horizontal column 5, table 2, the articular changes in the control and test animals are given irrespective of the age of the mice at the time of death. The number of animals showing normal joints was almost the same in both series (13.3 per cent in the controls and 9.9 per cent in the test animals). However, the incidence of age changes as well as that of osteoarthritis varied markedly in the two groups. Of the control animals 54.8 per cent disclosed articular age changes, while 31.9 per cent had osteoarthritis. In the test animals this proportion was practically reversed: 30.6 per cent disclosed articular age changes, while 59.5 per cent had osteoarthritis.

Irrespective of the age of the animals, the incidence of osteoarthritis was thus increased about twofold, an increase which from a statistical point of view is very significant (significant on the 1 per cent level).[7]

THE RELATIONSHIP BETWEEN ARTICULAR CHANGES AND AGE

Table 3 gives the number of animals and their mean ages grouped according to the findings in the joints at the time of death. The mean age of all animals with normal joints was 14.0 months in the control and 13.0 months in the test group

The mean age of all control mice with articular age changes was 16.2 months and of those showing osteoarthritis 20.2 months. The corresponding ages of the test animals were 13.7 and 17.8 months, respectively. Thus in control as well as in test animals the difference between the mean ages of mice with age changes and mice with osteoarthritis was about the same (four months for the controls and 4.1 months for the

TABLE 3.—*The Relationship Between the Articular Findings and the Mean Age of the Animals*

Experimental Group	Mice	Mice Showing No Change			Mice Showing Age Change			Osteoarthritis		
		No.	% of Total	Age, Mo.	No.	% of Total	Age, Mo.	No.	% of Total	Age, Mo.
Control	113	15	13.2	14.0	62	54.8	16.2	36	31.9	20.2
Test	111	11	9.7	13.0	34	30.6	13.7	66	59.5	17.8

test animals). On the other hand, mice fed the high fat diet showed articular age changes at a mean age of 13.7 months, whereas the mean age of the controls showing articular age changes was 16.2 months. Under the influence of the high fat diet articular age changes were thus advanced by 2.5 months. A similar difference of 2.4 months was observed between the mean ages of mice showing osteoarthritis (17.8 months for the test mice and 20.2 months for the controls). This difference in the acceleration of the onset of osteoarthritis is very significant statistically. However, the high fat diet caused no further acceleration of the course of osteoarthritis beyond the degree to which the preceding age changes had been hastened.

RELATIONSHIP BETWEEN ARTICULAR CHANGES AND WEIGHT

In table 4 the findings in the joints are given in relation to the mean weights of the animals. Animals over 18 months of age and obviously sick animals, as well as animals whose weights had dropped below 25 Gm., were not included in the computation, because unrelated diseases

7. The statistical analysis of these and the subsequent data was made by Prof. V. W. Lemmon, of the department of psychology of Washington University.

or undernourishment are known to influence the course of articular changes.[9b,c] The maximum weights served as the basis for the tabulation. Consecutive vertical columns show the mean weights of all control and test animals 9 to 18 months old whose joints were unchanged, or disclosed age changes or osteoarthritic lesions.

In the control series the mean weights of animals with normal joints and of those with age changes did not differ much (29.3 and 30.0 Gm., respectively, in the younger group, and 33.3 and 32.9 Gm., respectively, in the older group). In both age groups the animals showing osteoarthritis had higher mean weights than animals showing normal joints or simple age changes: At 9 to 12 months of age the mice with osteoarthritis weighed 33.0 Gm.; that is 3.7 Gm., or about 10 per cent, more than those showing normal joints. At 13 to 18 months of age the mean

TABLE 4.—*Findings in the Joints and Their Relation to the Mean Weight of the Animals*

			Mice Showing No Change			Mice Showing Age Change			Mice Showing Osteoarthritis		
				Deviation, Gm.			Deviation, Gm.			Deviation, Gm.	
Age, Mo.	Experimental Group	Mice	Mean Wt., Gm.	Maximum	Minimum	Mean Wt., Gm.	Maximum	Minimum	Mean Wt., Gm.	Maximum	Minimum
9-12	Control	31	29.3	0.7	2.3	30.0	2.0	4.0	33.0	1.0	4.0
	Test	29	35.8	8.2	0.8	35.7	5.3	3.7	40.6	12.4	10.6
13-18	Control	32	33.3	1.7	4.3	32.9	6.1	4.9	34.7	1.3	3.7
	Test	38	32.5	0.5	0.5	35.6	5.4	5.6	41.2	23.8	9.2
9-18 combined	Control	63	31.6	3.4	3.6	31.3	7.7	3.8	34.3	4.7	5.3
	Test	67	34.1	7.9	7.1	35.6	5.4	3.6	41.1	23.8	11.2

weight of mice with osteoarthritis was 34.7 Gm.; that is 1.4 Gm., or about 4 per cent, more than those showing normal joints. If all control animals are considered together, irrespective of their age, it is evident that the mean weight of the mice showing osteoarthritis (34.3 Gm.) was 2.7 Gm., or about 8 per cent, higher than the mean weight of those with normal joints (31.6 Gm.). Statistically, this difference is moderately significant (significant at the 5 per cent level).

Unlike the controls, the test animals showing age changes had a higher mean weight than animals with normal joints (35.7 Gm., compared with 33.8 Gm., in the younger group; 35.6 Gm., compared with 32.5 Gm., in the older group). These weight differences were, however, slight as compared with those existing between the test mice showing normal joints and those showing osteoarthritis (33.8 Gm., compared with 40.6 Gm., in the younger group; 32.5 Gm., compared with 41.2 Gm., in the older group; 34.1 Gm., compared with 41.1 Gm. for all mice irrespective of their age). The latter difference is statistically significant above the 5 per cent level.

COMMENT

Articular changes occurring spontaneously with advancing age in the knee joints of mice have been described previously.[4,7] These changes consist of growth and regressive processes in the cartilage, fibrinoid degeneration, metaplastic cartilagination and ossification in the ligaments, and proliferation and hyalinization in the membrana synovialis involving bone and bone marrow. While usually the changes in the cartilage predominate, there are instances in which the synovialis is affected without or with only slight participation of the articular cartilage. A combination of advanced growth and regressive changes of the various articular structures results in lesions which may be considered as an analog of human osteoarthritis.

The incidence, the onset and the severity of these changes vary with sex and strain, and they may be modified by administration of hormones or by removal of endocrine glands.[8b] In our present series of male mice of strain C57 black reared on a stock diet containing 5 per cent fat, 31.9 per cent showed osteoarthritis and 54.8 per cent slight or advanced age changes, whereas 13.3 per cent remained free of articular lesions, even in old age. Of male mice of the same strain receiving a diet containing 29 per cent fat, 59.5 per cent had osteoarthritis, 30.6 per cent age changes and 9.9 per cent unchanged joints. Thus, under the influence of the high fat diet there was an almost twofold rise in the incidence of osteoarthritis. This increase occurred as the result of a shifting away from simple age changes to the lesions of osteoarthritis. The present series of experiments thus shows a continuation of the trend established in growing mice fed the high fat diet up to 6 months of age: The joints of these animals had undergone premature aging and at the age of 6 months presented changes which in controls do not usually develop before the end of the first year of life.[1]

Age changes in the articular tissues may therefore be considered as forerunners and possibly pacemakers of osteoarthritic lesions. On the other hand, the number of mice with unchanged joints was about the same (13.3 per cent in the controls and 9.9 per cent in the test animals) no matter which ration was fed. Thus, a certain number of mice seemed to be resistant to the development of articular changes even in old age and even though the animals were exposed to stimuli which would call forth such changes in predisposed individuals.

The test animals not only had a higher incidence of osteoarthritis but had the disease at an earlier age (17.8 months) than the controls (20.2 months). This age difference of 2.4 months is practically identical with the difference in the ages at which simple age changes were found in the two groups of animals (13.7 months in the test group, compared with 16.2

months in the controls). Thus, in the test mice the mean age at which osteoarthritis was seen was advanced by only as much time as the simple age changes had been. The high fat diet, therefore, does not seem to play a role in the transformation of age changes leading to severe degenerative joint disease, but it apparently exerts its most evident effect on the progress of the early age changes. By accelerating the latter, the high fat diet may render the articular tissues more and prematurely susceptible to other injurious stimuli which contribute to the ultimate production of osteoarthritic lesions. These additional stimuli must be unrelated to the conditions of the present experiment, since a number of control mice likewise presented degenerative joint disease.

In view of the possibility that mechanical factors play a role in the etiology of osteoarthritis [8] and because overweight may exert excessive mechanical stress on the joints, the relationship between weight of the animals and articular lesions may be briefly discussed. If overweight should actually have any influence on the articular tissues, then not only the degree of overweight but also the length of time during which it is maintained as well as the age at which it occurs might be of importance. Control animals showing osteoarthritis had a mean weight of 34.3 Gm., whereas the test animals with normal joints had a mean weight of 34.1 Gm. Thus, weight as such is not an essential factor in the initiation of the articular changes. Moreover, individuals of the same weight showed different types of articular changes, while animals disclosing similar articular lesions differed widely in their weights. However, within both the control and the test groups osteoarthritis was associated with a tendency toward higher weights. This statistical coincidence was of moderate significance only. It does not necessarily prove an etiologic relationship of overweight and osteoarthritis but may be interpreted as independent responses of body weight and articular tissues, respectively, to the dietary regimen.

The mode of action of the fat-enriched diet on the articular tissues is as yet unknown. One of the major questions arising from the present investigation is the one regarding the specificity of the results. Our present experimental diet contained 6.0 calories per gram, compared with 4.2 calories per gram of the stock diet. A few preliminary observations indicate that, while the ad libitum consumption of individual mice fed the high fat diet varies considerably, the test mice consume at least

8. Payr, E.: Gelenksteifen und Gelenkplastik, Teil I, Berlin, Springer-Verlag, 1934. Bennett, G. A.; Waine, H., and Bauer, W.: Changes in the Kneejoints at Various Ages with Particular Reference to the Nature and Development of Degenerative Joint Disease, Commonwealth Fund, New York, Oxford University Press, 1942. Hench, P. S.; Bauer, W.; Boland, E. W.; Crain, D. C.; Freyberg, R. H.; Graham, W.; Holbrook, W. P.; Lockie, L. M.; McEwen, C.; Rosenberg, E. F., and Stecher, R. M.: Ann. Int. Med. **28**:66-168 and 309-451, 1948.

as many, and usually more, calories than the control animals. Our results may thus be due either to the increased caloric intake as such or to a specific action of the fat, or to both.

It is likewise undecided, whether the skeletal effects of the high fat diet are direct ones acting on cells or matrix or both, or whether they are mediated through changes in the general metabolism. These questions will have to be answered with the help of quantitative and histochemical methods.

SUMMARY

In male mice of strain C57 black fed throughout life a diet containing 29 per cent fat, articular aging was hastened as compared with that of controls fed a stock diet containing 5 per cent fat. Under the influence of the fat-enriched diet the incidence of osteoarthritis was increased two-fold and the onset of the disease significantly accelerated. As the incidence of osteoarthritis increased, that of simple age changes decreased. Articular age changes may, therefore, be considered as precursors of, and possibly pacemakers for, the severe lesions of degenerative joint disease. A number of animals remained free of articular changes even in old age and even if fed the fat-enriched diet. Under the conditions of the present experiment the overweight associated with the consumption of the high fat diet played only a minor role, if any, in the pathogenesis of degenerative joint disease.

Silberberg-'50

Bib No. 497

Federat. Proceed. 9:343-344. 1950.

Sex differences in skeletal development of mice fed a high fat diet. MARTIN SILBERBERG AND RUTH SILBERBERG. *Snodgras Lab., City Hospital, and Dept. of Pathology, Washington Univ. School of Medicine, St. Louis, Mo.*

In growing mice, the course of the skeletal time curve could be accelerated and articular age changes produced prematurely by feeding a ration of Purina Laboratory Chow with 25% lard added. This diet contains adequate amounts of protein, carbohydrates, minerals and vitamins. Since the female skeleton develops more rapidly and responds to hormonal stimulation less conspicuously than the male skeleton, it seemed interesting to compare the skeletal effects of the high fat diet in both sexes. Twenty six virgin females of strain C57 black were fed the high fat diet from the time of weaning on and killed together with 26 controls fed the stock ration after ½, 1, 2, 3, or 5 months' observation. The females fed the high fat diet showed better gain in weight and, histologically, faster epiphyseal development and earlier onset of articular age changes than controls fed the stock diet. However, the differences between the 2 groups of females were less pronounced than those occurring in corresponding groups of males. The weaker reaction of the female may be due to sex differences in food intake or to the comparatively more advanced physiological age of the female skeleton at the beginning of the experiment. Whatever the mechanism involved, because of their weaker response females are less suited than males for studies concerning effects of nutritional factors in skeletal growth and ageing.

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Am. J. Physiol. 177:23-26. 1954.

Silberberg-'54
Bib No. 498

Factors Modifying the Lifespan of Mice[1]

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THE REACTION of the skeleton of mice as well as the response of their body weight to changes in dietary regimen have been studied previously (1). The results varied with the strain, age, and sex of the animals used. The present report deals with observations on the lifespan of these mice. The data are presented because of the paucity of similar information available, even though the experiments were originally not designed for the purpose of longevity studies (2).

MATERIAL AND METHODS

Four hundred and sixty-three mice born at all seasons and raised in our laboratory were observed. Two hundred and five males and 130 virgin females belonged to the closely inbred strain C57BL, while 128 males were of the closely inbred strain DBA. From the time of weaning, 100 males and 76 females of strain C57, and 74 males of strain DBA were fed a stock diet of Purina Laboratory Chow containing 5% fat, while 105 males and 54 females of strain C57, and 54 males of strain DBA received a diet containing 20% fat. The latter ration was prepared by adding 24% lard (Swift's Silverleaf Brand) to the Laboratory Chow which had been ground to a fine meal. Details of the composition of this diet which proved to be adequate in all respects have been given previously (1). The animals were caged in groups of 5–7 to a cage.

Since the experiments were primarily planned for the study of skeletal changes, a number of animals had to be killed at predetermined ages, whether they were sick or not. The number of these animals varied in various groups; they were the fewer, the more animals had died spontaneously or had to be killed because they were in poor shape. Animals killed according to schedule were not included in the present paper. The most frequent single cause of death or illness, as far as could be determined at necropsy, was chronic kidney disease and anemia. Occasional instances of pneumonia were observed as well as a few tumors, especially hepatomas or lymphomas.

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Details of the distribution of the animals in the various groups can be seen from table 1.

OBSERVATIONS

Weights. Tables showing the weights of male mice of strains C57 and DBA fed the stock or high fat diet respectively have been published previously (1). Briefly, C57 males fed the stock diet reached their maximum mean weights of 32.3 gm at the age of 15 months; the highest individual weight observed was 40 gm in a mouse 21 months old. The maximum mean weight of C57 males fed the high fat diet was 40.1 gm at 22 months of age. This constitutes a 25% increase over the maximum mean weight seen in males of this strain fed the stock diet. The highest individual weight observed in the group receiving the high fat diet was 65 gm at 15 months of age. Deviations from the means were conspicuous in males fed the fat-enriched diet; they amounted to as much as +28.9 gm; in stock-fed males the widest deviation noted was +8.6 gm.

Males of strain DBA kept on the stock diet had their maximum mean weights of 23.8 gm at 17 months of age. The highest individual weight was 27 gm at the age of 15 months. The maximum mean weight seen in DBA males fed the high fat diet was found at 11 months of age; it was 24 gm and differed therefore immaterially from that seen in the corresponding mice fed the stock diet. In DBA males receiving the high fat diet, the highest individual weight, 28 gm was noted at 18 months of age. The maximum deviations from the mean reached +5.8 gm in stock-fed and +6.1 gm in the high-fat fed mice.

The increase in weight under the influence of the high fat diet was therefore much more marked, and the deviations from these means were much more conspicuous in C57 males than in DBA males.

The weights of C57 females took a position intermediate between that of the C57 males

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and DBA males. In stock-fed females, the maximum mean weight of 26.6 gm was seen at 18 months of age. The highest individual weight observed was 30 gm, and it was noted in several animals at various ages. The mice fed the high-fat diet reached a maximum mean weight of 30.6 gm at the age of 16 months. This represents an approximate increase of 15% over the maximum mean weight of the stock-fed females. The highest individual weight was 40 gm, recorded at 14 months of age.

TABLE 1. DISTRIBUTION OF ANIMALS AS TO SEX, STRAIN AND DIET FED

	Strain C57BL				Strain DBA	
	Stock Diet		High-Fat Diet		Stock Diet, Males	High-Fat Diet, Males
	Males	Females	Males	Females		
Total no. of animals observed	100	76	105	54	74	54
No. of animals killed in samples, as scheduled	46	19	50	19	0	0
No. of animals dead or killed because of poor condition and included in this report	54	57	55	35	74	54

Lifespan. In table 2, the mortality rates and the mean ages of the mice of the various groups at 100 days' intervals are presented. Table 3 was prepared in order to point out the ages at which 25, 50, 75, or 100% of the animals of the various groups were dead.

The following conclusions may be drawn from the data presented in tables 2 and 3: Feeding of the high fat diet shortened the lifespan of C57 males and increased their mortality. The mean lifespan of the mice fed the high fat diet was 144 days (16.1%) shorter than that of the stock-fed mice, and in the former a 50% mortality was reached 123 days sooner than in the latter. The mortality of stock-fed C57 females was higher than that of stock-fed males of the same strain, but the lifespan of the females was not adversely affected by the fat-enriched diet.

The mean lifespan of stock-fed DBA males was 157 days (21.9%) shorter than that of C57 males. In DBA males a 50% mortality was observed 192 days earlier than in C57

TABLE 2. MORTALITY OF MICE OF VARIOUS GROUPS AT 100 DAYS' INTERVALS

Age	C57♂ Stock Diet, 54 Animals		C57♂ High Fat Diet, 55 Animals		C57♀ Stock Diet, 57 Animals		C57♀ High Fat Diet, 35 Animals		DBA♂ Stock Diet, 51 Animals		DBA♂ High Fat Diet, 51 Animals	
	Mortality	Mean age (range)	Mortality	Mean age (range)	Mortality	Mean age (range)	Mortality	Mean age (range)	Mortality	Mean age (range)	Mortality	Mean age (range)
days	%	*days*	%	*days*	%	*days*	%	*days*	%	*days*	%	*days*
300	2	228							9	288	18	266
400	2	228	18	369	7	381	6	304	31	352	48	373
500	11	409	36	413	26	411	20	431	53	380	76	392
600	28	427	58	461	44	485	40	492	90	452	96	406
700	67	588	91	528	79	538	80	569	99	470	100	416 (210–656)
800	85	617	100	546 (301–730)	100	586 (364–735)	97	601	100	474 (269–717)		
900	100	653 (228–874)					100	606 (392–803)				

TABLE 3. AGES AT WHICH 25, 50, 75, AND 100% OF THE ANIMALS WERE DEAD

Animals Dead	C57♂ Stock Diet		C57♂ High Fat Diet		C57♀ Stock Diet		C57♀ High Fat Diet		DBA♂ Stock Diet		DBA♂ High Fat Diet	
	Max. age	Mean age	Max. age	Mean age	Max. age	Mean age	Max. age	Mean age	Max. age	Mean age	Max. age	Mean age
%	*days*		*days*		*days*		*days*		*days*		*days*	
25	557	481	438	382	486	437	547	456	355	321	311	279
50	604	559	541	447	607	501	621	519	472	373	415	325
75	717	603	637	499	661	542	672	565	551	427	472	308
100	874	653	730	546	735	586	803	606	717	474	656	416

males living under the same environmental conditions. Feeding of the high fat diet shortened the mean lifespan of DBA males by only 61 days (9.1%) as compared to 14.3% in C57 males. Similarly, in DBA males fed the high-fat diet a 50% mortality was reached 57 days earlier than in stock-fed controls, while this difference amounted to 123 days in males of strain C57. Therefore, the harmful effect of the high fat diet on the lifespan was far less marked in males of strain DBA than in males of strain C57.

DISCUSSION

The absolute lifespan of male mice of strain C57BL was about 22% longer than that of males of strain DBA. Moreover, both the mean and actual ages at which a given percentage of animals were dead were consistently higher in males of strain C57 than in those of strain DBA. Males of strain C57 outlived virgin females of the same strain. At any given age, virgin females showed a higher mortality than males of this strain. On the basis of our material the possibility cannot be ruled out that occasionally females might grow older than the maximum of 735 days at which our observations in this group were terminated. However, if one compares the ages at which 25, 50, or 75% of these animals were dead, these ages were invariably lower in virgin females than in males. In regard to longevity then, mice of strain C57BL did not follow the rule derived from observations of numerous species in which the females outlive the males. Exceptions from this rule have also been recorded in other mice, such as C57xDBA hybrids (3), strain LAF1 (4) and in the Syrian hamster (5).

Consumption of a high fat diet from weaning throughout life increased the mortality and appreciably shortened the lifespan of males of strain C57BL and DBA, as it did in rats (6). The harmful effect of the high fat diet on longevity was by far more conspicuous in individuals of the long-lived strain C57 than in those of the short-lived strain DBA. Apparently, mice of strain DBA are capable of metabolizing dietary fat with less injury to the organism as a whole than are mice of strain C57. Variations in the metabolism of dietary fat obviously extend to strains of mice other than the ones used by us, such as strain C3H (7). Endocrine influences may account for differences in the response to the high fat diet: The thyroids of mice of strain C57, for instance, are less active than those of strain DBA (1).

Virgin female mice of strain C57 showed a better tolerance to the high fat diet than males. This increased tolerance manifested itself in the response of body weight and of the skeletal tissues as well as in the duration of life. The mortality rate of virgin females fed the stock, or the high fat diet was about the same, the animals fed the fat-enriched diet showing perhaps a slightly lower mortality rate. Moreover, the oldest three mice fed the high fat diet outlived the oldest stock-fed animals by 68 days. However, because of the special arrangement of the experiment, the significance of this observation is difficult to evaluate. Still, the long survival of these mice is consistent with the comparatively low mortality rate of the entire group. The high fat diet, therefore, failed to exert an injurious effect on the lifespan of virgin female mice as it did in males.

The adverse effect of overweight on the lifespan is being increasingly recognized (8, 9). According to the findings in our mice it would also appear that the most marked shortening of the lifespan is associated with the most conspicuous increase in body weight. While this is true in a statistical sense, there was no consistent correlation between overweight and length of life of individual animals. Some of the heaviest C57 males lived beyond the age at which 50% of the animals had died. The injurious effect of the high fat diet on longevity seems, therefore, to be at least partly independent of overweight.

Age plays a prominent role in determining the tolerance to the fat-enriched diet. Males of strains C57 fed the high fat ration from the age of 200 or 365 days on showed a better tolerance to the high fat diet and much less gain in weight than males receiving this diet from the time of weaning on (10). This age effect may be tied to physiological rather than to chronological age. The strain differences in the response to the high fat diet may then be related to the fact that mice of strain DBA are at any given chronological age physiologically older than corresponding mice of strain C57. These early observations are presently being supplemented by additional experiments.

SUMMARY

Male mice of strain C57BL outlived males of strain DBA. Feeding of a high-fat diet throughout life appreciably shortened the lifespan of mice of both strains, but this effect was more marked in males of strain C57 than in males of strain DBA. The mortality rate of virgin females of strain C57 was higher than that of C57 males. Feeding through life of a high fat ration to virgin females of strain C57 did not shorten the lifespan of these animals. The injurious effect of the fat-enriched diet on longevity seems to be at least partly independent of the overweight observed in some of these mice.

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GROWTH AND ARTICULAR CHANGES IN SLOWLY AND RAPIDLY DEVELOPING MICE FED A HIGH-FAT DIET

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In mice of strain C57 black fed a high-fat diet throughout life, the skeletal time curve was accelerated: As compared with the corresponding processes in mice kept on a regular diet, growth and development of the long bones were hastened, articular changes set in prematurely, and the incidence of osteoarthritis was increased. Males reacted more readily and more intensely than females. This sex difference was tentatively explained, as follows: The male skeleton ages more slowly than the female; moreover, the susceptibility of the skeleton to stimuli decreases with advancing age, a condition observed previously regarding the skeletal effects of hormones (Silberberg and Silberberg 1943, 1949, 1950).

If this interpretation was correct, the high-fat diet should modify skeletal growth and ageing less conspicuously in individuals of rapidly developing strains of mice, such as dba, than in those of slowly developing strains, such as C57 black. In order to further analyze the relationship between the rate of physiologic development and ageing and the skeletal response to stimuli, the following experiments were carried out.

MATERIAL AND METHODS

One hundred and ninety-five male mice of the closely inbred strain dba, raised in our laboratory, were used. The animals were four to five weeks old and weighed on the average 12 grams at the beginning of observations. *Control Series:* One hundred and six mice were fed a stock diet of Purina Laboratory Chow as libitum with

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water available at all times. This ration contains 5% fat. *Test Series:* Eighty-nine mice received a high-fat diet consisting of 75% laboratory Chow ground to a meal with 25% lard (Swift's "Silverleaf" Brand) added. This diet, details of which have been given previously, contains 29% fat (Silberberg and Silberberg 1950b); it is adequate in all respects and well tolerated by the animals. The mice were weighed at regular intervals. During the period of growth, mice of the control and experimental series were killed in groups of 4 or 6 each, after ½, 1, 2, 3, or 5 months of observation. Later on, samples of animals were sacrificed in 2 or 3 months' intervals. A number of mice were allowed to live to the end of their natural life, while others were killed, when their condition warranted it. According to their age at death, the following three age groups of adult animals were available for examination: Age group I: 7-12 months: 30 control and 25 test mice; age group II: 13-18 months: 40 control and 33 test mice; age group III: 19 months and over: 10 control and 5 test mice.

The material secured at necropsy was handled in the usual manner. The knee-joints including the lower end of the femur and the upper part of the tibia were removed as a whole, fixed and decalcified in Bouin's solution, and embedded in paraffin. Sectorial sections were stained with hematoxylin and eosin.

Gross Observations

The animals appeared healthy, and showed no signs of nutritional deficiencies. During the period of growth, the mean weights of the test animals lagged slightly behind those of the controls. However, at the age of six months, the mice of the two groups showed about equal weights. During the second year of life, the test animals weighed approximately 8% more than the controls. The individual weights of the test animals varied within a wider range than those of the controls. The following table gives the mean weights of the mice in the control and test series and the maximum and minimum deviations from these means.

Microscopic Examination

Growth Zones

Mice 1½ months old: In the controls, the width of the growth zones was about 165 microns, the single cartilage cell row being composed

TABLE 1
SHOWING THE MEAN WEIGHTS AND THE DEVIATIONS FROM THE MEANS IN CONTROL AND TEST ANIMALS

	Controls		Tests	
initial	12.2	max. d. 0.8; min. d. 2.2	12.4	max. d. 0.6; min. d. 2.4
2 months	15.2	max. d. 0.8; min. d. 1.2	15.3	max. d. 2.7; min. d. 0.3
3 months	17.2	max. d. 0.8; min. d. 1.2	16.2	max. d. 1.8; min. d. 1.2
4 months	19.2	max. d. 1.8; min .d. 1.2	18.6	max. d. 2.4; min. d. 1.6
5 months	19.2	max. d. 2.8; min. d. 0.2	17.9	max. d. 3.1; min. d. 0.9
6 months	19.3	max. d. 2.7; min. d. 0.3	19.6	max. d. 3.4; min. d. 1.6
7 months	19.2	max. d. 5.8; min. d. 3.2	20.5	max. d. 6.5; min. d. 5.5
8 months	19.2	max. d. 6.8; min. d. 2.2	21.3	max. d. 5.7; min. d. 3.3
9 months	19.1	max. d. 5.9; min. d. 4.1	21.0	max. d. 6.0; min. d. 5.0
10 months	21.0	max. d. 4.0; min. d. 3.0	20.9	max. d. 6.1; min. d. 4.9
11 months	21.3	max. d. 4.7; min. d. 2.3	24.0	max. d. 3.0; min. d. 8.0
12 months	21.8	max. d. 4.2; min. d. 2.8	22.3	max. d. 3.7; min. d. 5.3
13 months	22.3	max. d. 3.7; min. d. 3.3	21.6	max. d. 3.4; min. d. 4.6
14 months	22.1	max. d. 3.9; min. d. 2.1	22.5	max. d. 2.5; min. d. 5.5
15 months	22.1	max. d. 4.9; min. d. 1.1	24.0	max. d. 1.0; min. d. 6.0
16 months	23.3	max. d. 2.7; min. d. 7.3	24.0	max. d. 2.0; min. d. 5.0
17 months	23.8	max. d. 3.2; min. d. 0.8	22.0	max. d. 1.0; min. d. 1.0
18 months	22.6	max. d. 1.4; min. d. 2.6	24.0	max. d. 4.0; min. d. 3.0
19 months	23.2	max. d. 3.8; min. d. 3.2	24.0	max. d. 3.0; min. d. 2.0
20 months	22.0	max. d. 1.0; min. d. 1.0	22.0	max. d. 3.0; min. d. 5.0
21 months		— —	24.0	max. d. 1.0; min. d. 1.0

of 7-9 columnar and 3-4 hypertrophic cells. In the test animals, the growth zones were about 150 microns wide, and the individual cartilage cell row contained 6-8 columnar and 2 or 3 hypertrophic cells. The cartilage cell rows showed regular arrangement; calcification of cartilage and the amount of chondromucoid groundsubstance were about the same in both groups of animals. In the test series, the primary spongiosa was coarser than in the controls.

Mice 2 months old: The growth zones of the controls measured about 150 microns, and in the single cartilage cell row 6-9 columnar and 2 or 3 hypertrophic cells were present (Fig. 1). In mice of the test series, the growth zones were narrowed down to about 130 microns, and in the single cartilage cell row 5-7 columnar and 1 or 2 small cells of hypertrophic type were counted. Hyalinization of the matrix and replacement of cartilage by bone were farther advanced in the test animals than in the controls (Fig. 2).

Mice 3 months old: In the control animals the growth zones were about 115 microns wide, and in the single cartilage cell row 5-6 columnar and 2 or 3 hypertrophic cells were found. The growth zones of the test animals measured only 75-90 microns, and the number of cells in the single cartilage cell row had decreased to 4-6 small col-

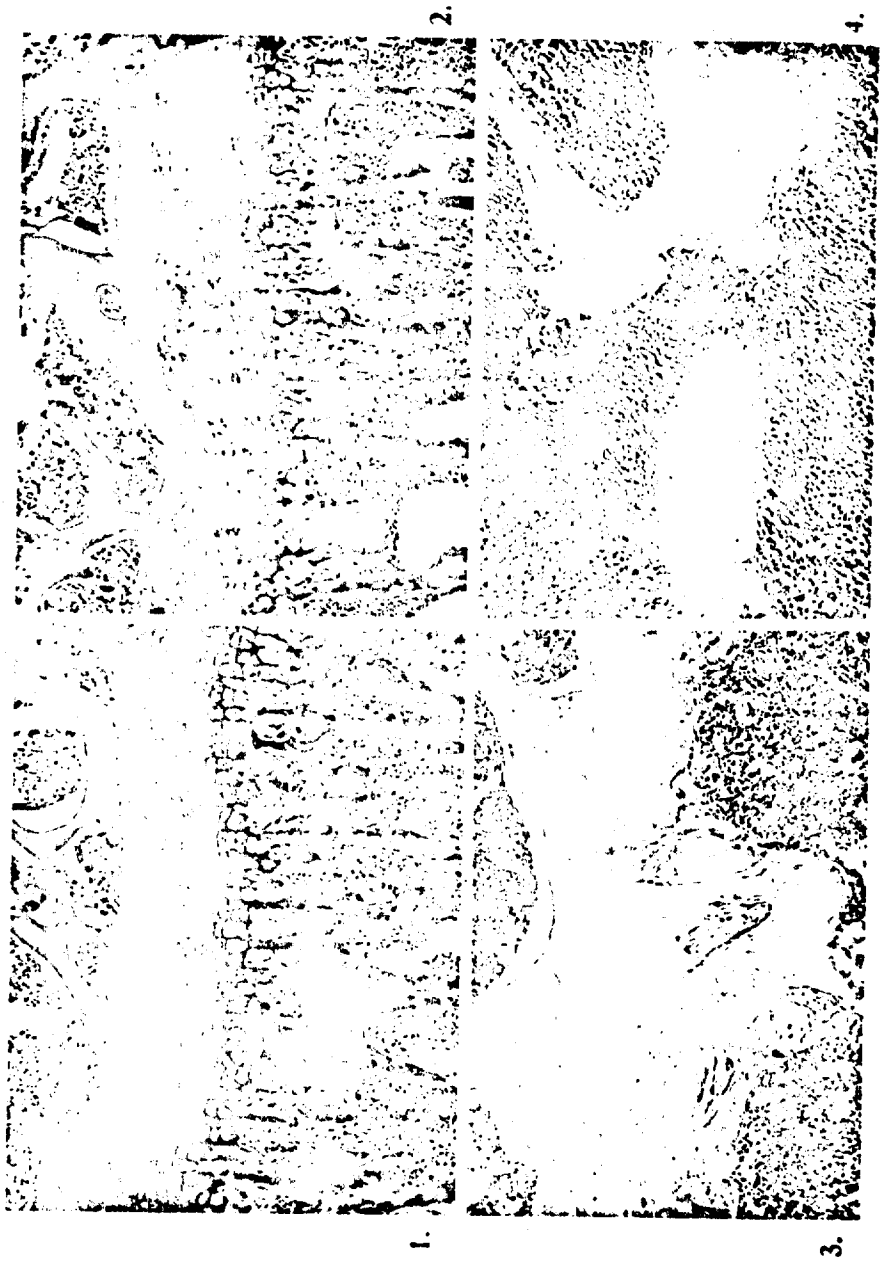
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PLATE I

Fig. 1-4. Sections through the growth zones at the upper end of the tibia of male mice of strain dba. x125.

Fig. 1. Two-month-old mouse fed the control diet. The cartilage cell rows are regularly arranged and separated from each other by thin layers of ground substance.

Fig. 2. Two-month-old mouse fed the high-fat diet since weaning. The growth zone is narrower, there are fewer hypertrophic cells and there is more ground substance between the cartilage cell rows, and the replacement of cartilage by bone takes place farther proximally than in the control.

Fig. 3. Six-month-old mouse fed the control diet. The epiphyseal cartilage is inactive and contains a thick osseous plug.

Fig. 4. Six-month-old mouse fed the high-fat diet. There is perforation of the inactive and partly ossified epiphyseal disk.

umnar and 1 or 2 small cells of hypertrophic type. In both groups of animals, regressive changes and destruction of cartilage had made progress, and ossified plugs of degenerated cartilage traversed the growth zone. Again these changes as well as metaphyseal and cortical bone formation were more accentuated in the test animals than in the controls.

Mice 4 months old: The growth zones of the controls were between 75 and 90 microns wide, and were composed of 5-7 small cells of columnar and hypertrophic type. The cartilage cell rows were separated from each other by large wedges of hyaline matrix and plugs of degenerated or ossified cartilage, that had increased in number and size as compared with the preceding stage. In animals of the test series, the growth zones were too irregular in width to be measured or to permit an exact cell count. Moreover, the distinction between columnar and hypertrophic cartilage became difficult. A transverse bony lamella indicative of cessation of growth delimited the epiphyseal cartilage from the metaphysis. The lamella as well as the cortical bone were thicker in animals of the test series than in the controls.

Mice 6 months old: In the controls, the growth zones consisted of sclerosed cartilage sealed off from the metaphysis by a bony plate Thick osseous plugs showing beginning resorption by bone marrow were present (Fig. 3). In the test animals, the remnants of the epiphyseal cartilage appeared more inactive than in the controls, and epiphyseo-diaphyseal union had already occurred (Fig. 4). In both groups of animals, vigorous resorption had led to a thinning out or to complete resorption of the primary spongiosa.

At still later ages, the structure of the growth zones of the controls approached gradually that of the test animals, and ultimately hardly any differences were noticeable in the two groups of animals.

Joints: The findings, representative sections of which are shown (Figs. 5-10), were graded in accordance with criteria fully described previously (15b). Briefly, this is the classification:

No Change: There is the usual development from undifferentiated to hypertrophic cells and replacement of cartilage by bone. The ligaments, synovialis and capsule show regular architecture.

Age Changes: Slight changes consist of focal or diffuse hyperplasia and hypertrophy of the cartilage cells, while hematoxylin-eosin stains fail to disclose regressive changes in the matrix. In other animals,

5.

6.

7.

8.

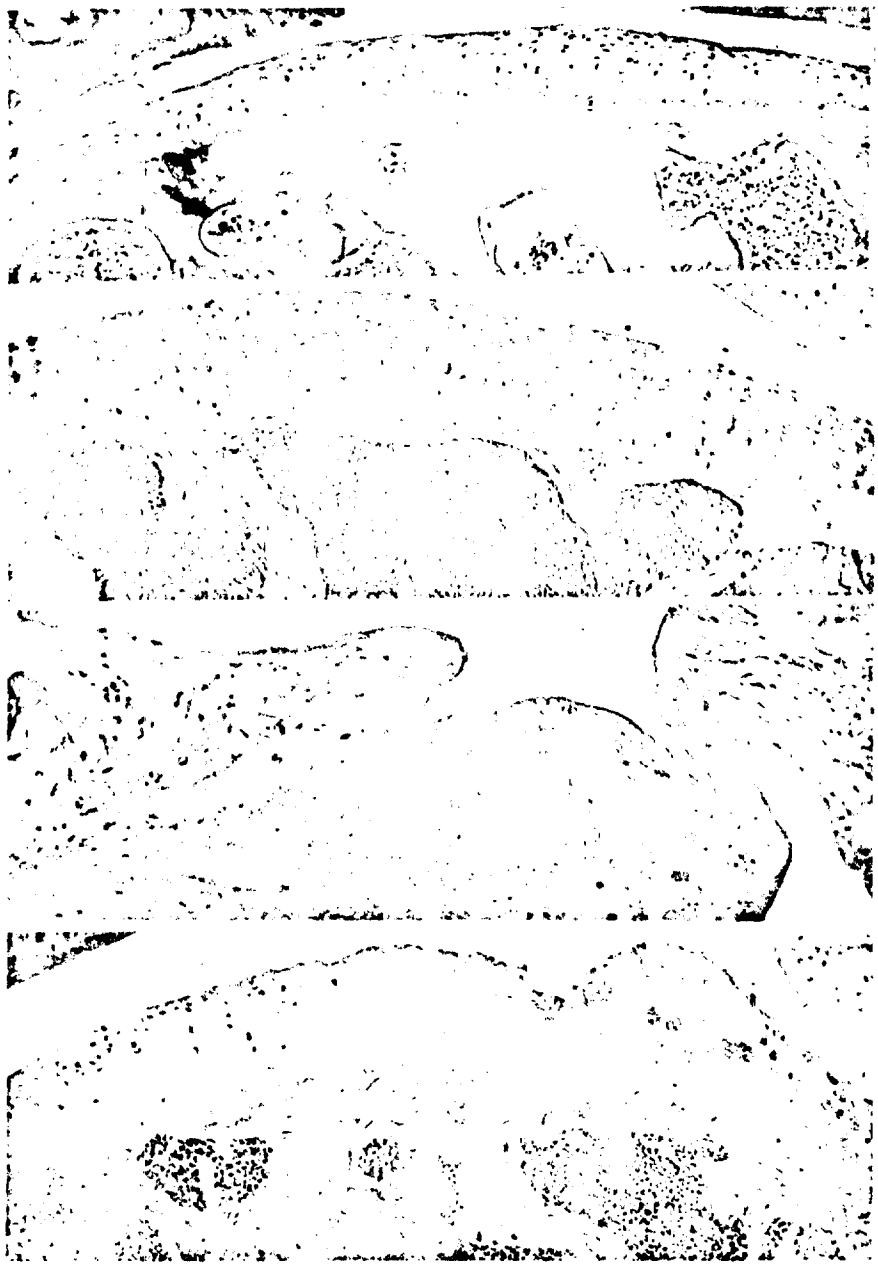


PLATE II

Fig. 5-8. Sections through the articular surface at the upper end of the tibia. x125.
Fig. 5. Resting articular surface.
Fig. 6. Articular surface showing proliferation of cartilage.
Fig. 7. Articular cartilage showing advanced growth.
Fig. 8. Ulcer at the surface with regressive changes in the cartilage at its edges.

the cartilage is resting, while the synovialis proliferates. The newly formed areolar tissue infiltrates into the epiphyseal bone marrow through enlarged vascular channels, and the ligaments undergo cartilagination.

Advanced age changes are characterized by marked proliferation or regressive changes in cartilage, ligaments or synovialis.

Osteoarthritis: There is a combination of growth and regressive changes associated with deformities of the joint: Focal or generalized growth of the cartilage, ulceration and eburnation of the articular surfaces, proliferation and fibrosis of the synovialis, fibrosis or cyst-formation of the bone marrow, cartilagination and degeneration of the lilgaments.

Results

Up to the age of six months, only slight and localized articular changes were found. In 3 of 12 controls, 4 and 6 months old, slight age changes were noted. In the test mice, joint changes were first seen at the age of three months: 3 of 18 animals, 3 to 6 months old showed slight, and 2 had developed advanced age changes.

Articular changes were seen more frequently from the second half of the first year on. The following table shows the grades and incidences of the articular findings in the adult animals.

At a mean age of 9.3 months more than one-half (56.0%) of the test animals had slight changes, but only about one-third (36.7%) of the controls—mean age 9.4 months—were thus affected. This increase had occurred at the expense of mice with resting joints, the latter being about three times as numerous (36.7%) in the controls as in the test series (12.0%). Towards the end of the first half of the second year of life, that is at the mean age of 16.1 and 17.6 months respectively, 51.5% of the test animals and 50.0% of the controls had developed slight age changes. Articular changes had thus remained stationary in the test animals but had made progress in the controls. In the animals 13 to 18 months old the high-fat diet had not significantly affected the course of degenerative joint disease. In the test mice, the incidence of advanced age changes was 9.1% and of osteoarthritis 33.3% as compared with a 12.5% incidence of advanced age changes and a 32.5 per cent incidence of osteoarthritis in the controls. In still older controls, the incidence of articular changes increased only slightly (20.0%) and osteoarthritic lesions

TABLE 2

SHOWING THE INCIDENCES OF THE ARTICULAR FINDINGS IN ANIMALS OF THE DIFFERENT AGE GROUPS

Age group months		Total No. of mice	Mean age at death months	No Change No. of mice	No Change %	Sight Age Change No. of mice	Sight Age Change %	Advanced Age Change No. of mice	Advanced Age Change %	Osteoarthritis No. of mice	Osteoarthritis %
7-12	Controls	30	9.4	11	36.7	11	36.7	5	16.6	3	10.0
	Tests	25	9.3	3	12.0	14	56.0	5	20.0	3	12.0
13-18	Controls	40	17.6	2	5.0	20	50.0	5	12.5	13	32.5
	Tests	33	16.1	2	6.1	17	51.5	3	9.1	11	33.3
19-21	Controls	10	19.7	1	10.0	5	50.0	2	20.0	2	20.0
	Tests	5	20.4	0	0.0	2	40.0	1	20.0	2	40.0
Irrespective of age	Controls	80	14.5	14	17.5	36	45.0	12	15.0	18	22.5
	Tests	63	14.0	5	7.9	33	52.4	9	14.3	16	25.4

even declined (20.0%). As had been observed in strain C57, there seems to be a peak in susceptibility to articular changes about late middle age. The 40% incidence of osteoarthritis in test mice over 19 months of age is inconclusive in view of the small number (5) of animals reaching this age.

A consideration of the various articular changes in controls and test mice irrespective of age discloses the following: Under the influence of the high-fat diet there occurred but a slight shift from unchanged joints to those with slight changes. The total incidence of osteoarthritis was the same in the control and test animals. The results thus support the view that the skeletal response to certain stimuli decreases with advancing physiologic age, and that the presence of age changes alone is not the only basis for the development of osteoarthritis. Apparently additional prerequisites must be fulfilled before the osteoarthritic changes take place.

Observations Concerning Strain Differences dba: C57 Black

The skeletal response to the high-fat diet of mice of strain dba is in striking contrast to results obtained in identical experiments carried out in mice of the slowly ageing strain C57 (Silberberg and Silberberg 1950b). In the latter, growth and development of the long bones were conspicuously accelerated, and the incidence of osteoarthritis increased twofold under the influence of the fat-enriched diet.

The strain differences are apparently not confined to the skeleton. As compared to controls fed a stock diet, mice of strain dba receiving the fat-enriched diet gained only slightly more weight—about 8%—than their controls, and showed hardly any increase in fat deposits, while in the corresponding animals of strain C57, there was as much as 100% gain in weight associated with the deposition of considerable amounts of body fat.

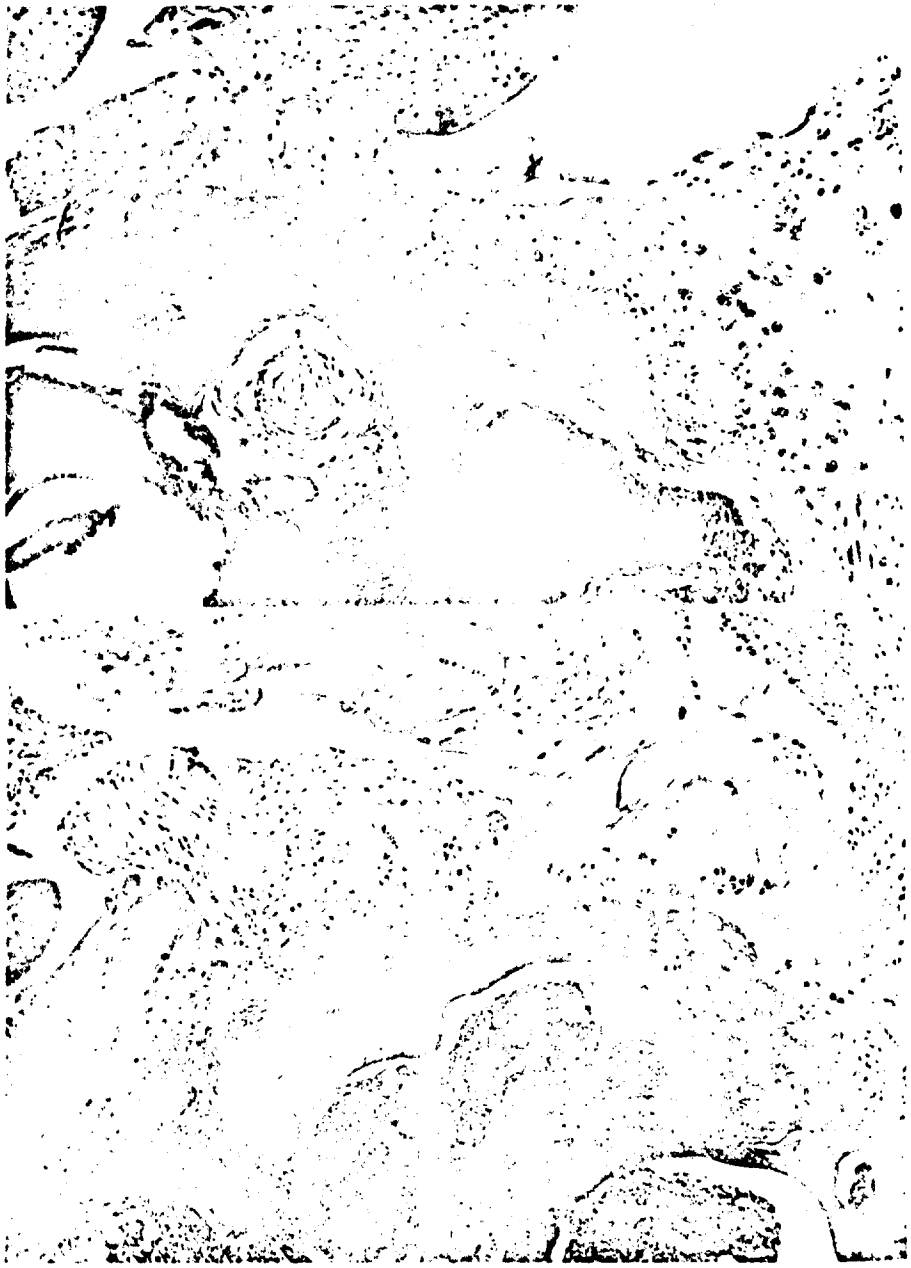
The two strains did not differ only in their reaction to the high-fat diet, but also in the microscopic appearances of the normal joints and in the development of spontaneous articular lesions. In strain dba, the bones were more delicate and the cartilage was less abundant than in strain C57. In the articular cartilage of adult and old mice of strain dba, growth was much less active and regressive changes were by far less extensive than in strain C57. In aged animals of the latter strain, ulceration of the articular surface with over

growth and eburnation were rather common, while this was a rare occurrence in strain dba. The synovialis on the other hand, reacted more conspicuously in the latter than in strain C57. In strain dba, joints showing primary synovial changes outnumbered those in which the cartilage was primarily altered. Conversely, in strain C57 joints with resting cartilage and proliferating synovialis were rare.

In addition, the thyroids of mice of strain dba showed signs of increased activity as compared with those of strain C57 (Figs. 11, 12). In the former, the acini were lined by high epithelium and contained softened scalloped colloid, while in the latter the acinar epithelium was cuboidal, and the colloid was dense without evidence of absorption. In adult mice of strain dba, the thyroids showed cysts filled with purplish secretions, an uncommon finding in strain C57 (Figs. 13, 14). Similar variations in the thyroid of mice of various strains have been noted by other investigators (Barry and Kennaway 1937, Loeb and Kirtz 1939, Grüneberg 1943, 1947).

Discussion

The present discussion has to revolve around two points: The strain differences in the spontaneous age changes, and those in the response to the high-fat diet. The first problem includes the question whether the relative resistance of the articular cartilage in mice of strain dba is a local phenomenon or whether it is controlled by extraarticular factors. The comparatively small amount of pre-existing cartilage in this strain may preclude an extensive growth response. The greater refractoriness of the cartilage may, however, also be due to differences in biochemical and biophysical composition. The latter will change in different strains depending on the rate of physiologic development and ageing which is in part genetically determined (Bauer and Bennett 1936, Loeb 1940, 1945, Silberberg and Silberberg 1943, 1949). Moreover, the composition of the cartilage, is probably also governed by extra-articular factors, one of them being the thyroid hormone. The appearance of the cartilage, the delicacy of the bony structures, and the scarcity of hyperostotic osteoarthritic lesions in mice of strain dba are consistent with increased activity of the thyroid. Thyroid hormone promotes resorption of bone; it also exerts a condensing effect on the matrix and might thus inhibit growth of cells as well as softening of the groundsubstance, both important steps in the pathogenesis of osteoarthritis. Estrogen, although inhibiting



9.

10

PLATE III

Fig. 5-10. Sections through the articular surface at the upper end of the tibia. x125.

Fig. 9. Osteoarthritic lesion at the surface of the upper end of the tibia with fibrosis of the bone marrow, proliferation of the cartilage, and cartilagination of the ligament. x150.

Fig. 10. Advanced osteoarthritis at the upper end of the tibia. x125.

11. 12.

13. 14.

PLATE IV

Fig. 11-14. Sections through the thyroid glands of normal male mice. x200.

Fig. 11 Mouse of strain C57 black, 2 months old. The acini are of usual size and contain hard colloid, the acinar epithelium is low cuboidal.

Fig. 12. Mouse of strain dba, 2 months old. The acini are large and lined by high epithelium, the colloid is soft and shows scalloping.

Fig. 13. Mouse of strain C57 black, 6 months old. The thyroid gland is resting and contains firm colloid, the acinar epithelium is low cuboidal.

Fig. 14. Mouse of strain dba, 6 months old. Large cysts with secretions are present.

resorptive processes, likewise causes condensation of the matrix (Silberberg and Silberberg 1943, 1949). However, this hormone is probably not involved in the present results, since males were used.

The observations of strain differences in the response to the high-fat diet may also help in the analysis of mechanisms involved in the spontaneous processes of ageing, the nature of which is still little understood. Thus, the differences in gain of body weight in the two strains point to differences in their fat metabolism. Possibly, mice of strain dba may be capable of metabolizing excessive fat, while mice of strain C57 are unable to do so. Reports concerning strain differences in various phases of metabolism appear in increasing numbers: Certain strains of rats vary regarding the lipotropic action of administered estrogen (György and Rose 1949). According to some investigators, mice with a high rate of mammary cancer—like strain dba—produce larger amounts of estrogen than do lower cancer strains such as strain C57 (Korteweg 1948). However, in adult females of strain dba, the slender build of the skeleton did not indicate hyperestrinization. In this strain thus—and possibly in contrast to the high mammary cancer strain C3H—the high breast cancer incidence may be due to increased tissue susceptibility rather than to increased production of estrogen. Moreover, various strains of mice differ in their requirements for vitamins (Fenton and Cowgill 1947), in their turnover of phosphorus (Johnson and Albert 1949) and iodine (Gross and Schwartz 1950), and in the lipid fractions contained in their adrenals (Vicari 1943, Vicari and Little 1945). Since thyroid hormone may act as a controlling agent in fat transfer (Handler 1948), increased thyroid activity in mice of strain dba may account for rapid breakdown of fat consumed in excess. Finally, vitamin A plays a rôle in both the metabolism of fat and in skeletal development (Eddy and Dalldorf 1944, Maddock, Wolbach and Maddock 1949) and also is closely related to thyroid activity (Heimer, Maslow and Sobel 1949). This vitamin may thus be likewise involved in both the spontaneous and the experimental strain differences, although its exact position in the chain of events is as yet unknown.

Summary

In mice of strain dba the long bones show a more delicate build, and the skeletal time curve is accelerated as compared with conditions

in strain C57 black. The two strains differ also in the structure of various endocrine glands. Differences in thyroid activity may be responsible for some of the skeletal strain characteristics and for variations in the skeletal time curve.

In mice of strain dba, skeletal growth and ageing were only slightly hastened, and the incidence of articular lesions was unaffected by feeding a fat-enriched diet throughout life. These results are considered to be related to local qualitative and quantitative differences in the composition of the cartilage, to differences in the rate of physiologic development and ageing, and to increased thyroid activity exerting its effect not only directly on the cartilage but also on the metabolism of fat in general.

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Silberberg-'55
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LIFE SPAN OF MICE FED A HIGH FAT DIET AT VARIOUS AGES[1]

BY RUTH SILBERBERG AND MARTIN SILBERBERG

Abstract

The effect of a high fat diet on life span depends on strain and sex of the animal tested and is specifically due to the fat content, and not to the high caloric value, of the diet. In male mice of strain C57BL, feeding of a high fat diet from the ages of 1, 6, or 12 months on through life shortened the life span. If the high fat diet was given for a limited period of time, the effect on life span depended on the age at which these mice were placed on the high fat ration.

Feeding a diet containing 30% fat to male mice of strain C57BL from the time of weaning through life significantly shortened the life span of these animals as compared to that of controls fed a stock ration containing 5% fat (6). In continuation of these investigations, it was thought of interest to record the effect on life span of a fat-enriched diet given at various ages and for different periods of time.

Material and Methods

Four hundred and forty-one male mice of the closely inbred strain C57BL raised in our laboratory were observed. One group of animals fed the stock diet of Purina Laboratory Chow through life (group I) and another group fed the high fat diet from weaning through life (group II) served as controls. Five groups of mice were given the high fat diet for limited periods of time. In two of these groups, the fat-enriched ration was fed to the mice from the age of six months (group III) and 12 months (group IV) on through life; to the remaining three groups of animals, the diet was given for a period of five months starting at the ages of one month (group V), seven months (group VI), or 12 months (group VII), respectively. Up to the beginning and on through life from the end of these five months' periods, the animals received the stock diet. Both rations were given ad libitum with water available at all times. The distribution of the animals in the various groups is shown in Table I.

The high fat ration was prepared by grinding the Laboratory Chow to a meal and adding 25% lard (Swift's Silverleaf Brand). The exact composition of this diet, which is adequate in all respects and which is well tolerated, has been given previously (5).

Since the experiments were primarily designed for the study of skeletal changes, a number of animals were sacrificed at predetermined ages. The latter mice have been disregarded in the present analysis, and only those 225 animals that died or had to be killed because of sickness have been included

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Contribution from the Snodgras Laboratory, St. Louis City Hospital Division, and the Department of Pathology, Washington University, School of Medicine, Saint Louis, Missouri.

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TABLE I

DISTRIBUTION OF ANIMALS IN THE VARIOUS GROUPS

	Stock diet	High fat diet					
		From 1 month through life	From 6 months through life	From 12 months through life	From 1 to 6 months	From 7 to 12 months	From 12 to 17 months
	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Total number of mice	100	76	61	39	65	51	49
Number of mice killed as scheduled	46	19	33	16	42	30	30
Number of mice dead or killed because of sickness and included in this report	54	57	28	23	23	21	19

(Table I). The most frequent anatomical cause of death was chronic kidney disease and anemia; occasionally liver tumors, leukemia, or pneumonia were observed.

Observations

Weights

The weights of mice fed the high fat diet from 1, 6, or 12 months on through life have been reported and discussed previously (5). The body weights of the animals receiving the high fat diet were in most cases higher than those of the stock-fed animals. The differences were the more marked the longer the fat-enriched diet had been consumed. In mice fed the fat-enriched diet for five months, the increase in mean weight was least striking, and of temporary character; it never exceeded 10% and was usually closer to 5% above that of the stock-fed animals. Mice fed the high fat diet from the age of 12 months on showed an only insignificant gain in weight. Following discontinuation of the high fat ration, the weights returned to normal and showed the usual decline with advancing age.

Life Span

In Table II, the mortality and the mean ages of the mice in the various groups at 100 days' intervals are presented, and in Table III, the mean and the maximum ages at which 25, 50, 75, or 100% of the animals were dead are shown.

TABLE II

MORTALITY OF ANIMALS OF THE VARIOUS GROUPS AT 100 DAYS' INTERVALS

	Stock diet	High fat diet					
	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Age, days	Mortality, %	Mortality, %					
300	2		4				
400	2	18	11		13		10
500	11	36	14	26	17		16
600	28	58	40	43	22	29	32
700	67	91	93	74	48	62	63
800	85	100	100	100	82	71	79
900	100				95	90	100
1000					100	100	

* *Since less than 100 animals were available in each group, only the full percentages are given.*

TABLE III

AGES AT WHICH 25, 50, 75, AND 100% OF THE ANIMALS WERE DEAD

Mice dead, %	Stock diet		High fat diet											
	Group I		Group II		Group III		Group IV		Group V		Group VI		Group VII	
	Age, days		Age, days											
	Max.	Mean	Max.	Mean	Max.	Mean	Max.	Mean	Max.	Mean	Max.	Mean	Max.	Mean
25	557	481	438	382	540	424	486	433	606	456	584	541	538	458
50	664	559	541	447	663	507	628	491	709	554	656	587	646	524
75	717	603	637	449	696	567	713	557	719	607	817	635	743	584
100	874	653	730	546	701	600	722	593	972	655	993	694	879	643

Fig. 1 demonstrates the mortality curves of the mice of the various groups.

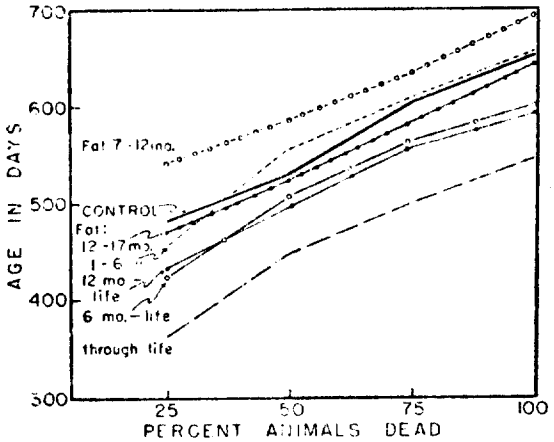

FIG 1. Mortality curves of animals fed the stock or high fat diet for varying lengths of time.

Feeding of the High Fat Diet Throughout Life

The maximum age of mice fed the high fat diet on through life from the time of weaning (group II) was 144 days (16.1%) lower and the mean life span was 107 days (16.4%) shorter than that of the stock-fed animals (group I). Similarly in the group consuming the high fat ration, the 50% mortality was observed 112 days earlier than in the stock-fed control group.

Feeding the high fat diet from the ages of six months (group III) or 12 months (group IV) on and through life thereafter affected the life span of these animals less adversely than that of mice fed this ration from the time of weaning on (group II). Although in groups III and IV the maximum ages reached were 701 and 722 days respectively, that is, somewhat less than the 730 days attained by group II, the mean ages at death of all animals in groups III and IV were higher than in those of group II. As compared to stock-fed animals (group I), the mean life span of mice of groups III and IV was shortened by 8.1% and 9.2% respectively. Similarly, the median life span was 122 and 87 days longer in mice of groups III and IV than that of 541 days observed in group II, and in mice of groups III and IV, the 50% mortality was reached at a mean age of 507 and 491 days respectively as compared to a corresponding 447 days in group II. At the 25 and 75% levels of mortality, the same relations between groups II, III, and IV prevailed as at the 50 and 100% levels.

Feeding of the High Fat Diet for Five Months at Various Ages

The oldest individual of the group fed the fat-enriched diet from the age of one to six months (group V) outlived the oldest stock-fed mouse by 98 days. Otherwise the mortality of these mice was similar to that of the stock-fed animals. The high fat diet thus exerted no injurious effect on the life span of

mice of group V. In this series, however, the number of animals sacrificed on schedule was almost twice the number that died or were killed because of disease. When sacrificed, the animals were 18 and 24 months old respectively, the mean age being 591 days, that is 37 days higher than that of 50% of the mice of this group that died or were or killed because of sickness. Therefore, the mean life span of these mice might have been longer than indicated in the table, if all individuals had been allowed to live to the end of their natural lives.

Mice fed the high fat ration for five months from seven months of age on (group VI), lived somewhat longer than stock-fed controls (group I). This prolongation of life was associated with a decreased mortality as indicated by consistently higher mean ages at the 25, 50, 75, and 100% levels of mortality. This difference in age was approximately one month except at the 25% level, where it amounted to 60 days. A selective factor of minor importance may have favored these results. Animals started on the high fat diet at seven months of age were taken from a stock the early mortality of which was not determined. The elimination of a few individuals through death before seven months of age might somewhat decrease the mortality rate of the surviving animals, although this factor should not materially influence the results.

Animals fed the fat-enriched diet from the age of 12 to 17 months (group VII) had the least favorable survival. However, the differences between this group and groups V and VI were slight amounting to one month at the 50% mortality level and declining thereafter. The oldest age reached and the mean age of all mice were about the same as those of the stock-fed controls. The effect of the high fat diet in mice of this group may, however, be more injurious than would appear from the figures in the table. The selective factor mentioned before eliminates mice dead before one year of age and presumably leaves in the experimental group individuals of greater resistance and thus better survival than characteristic of the colony in general.

Discussion

Feeding a high fat diet from the ages of 6 to 12 months on through life shortened the life span of male mice of strain C57BL, but did so to a lesser degree than consumption of the same diet through life from the time of weaning on (6). There was no appreciable difference in the mortality of the mice receiving the diet from the ages of 6 or 12 months respectively. Yet, mice started on the high fat ration at the age of 12 months (group IV) consumed this diet for six months less than mice receiving the same ration from the age of six months on (group III). Thus there are indications that old mice are more susceptible to the injurious effect of the high fat diet than young adult mice.

Feeding the high fat ration for a period of five months had only minor effects on the life span of mice thus treated. The ration was slightly injurious only if fed to mice which were past the age of one year. Again thus, old animals tended to be most susceptible to the adverse effects exerted on the life span by a high dietary level of fat. If the high fat diet was given during

the period of growth and shortly thereafter, the life span was not altered, and if fed in early adulthood, the diet even proved beneficial.

The latter results agree with findings in rats in which a certain degree of dietary enrichment with butter fat was beneficial (3, 4) or innocuous (1) as far as longevity was concerned. However, there seems to be a limit either as to the level of fat in the ration or as to the length of time during which the high fat ration is given beyond which injurious rather than beneficial effects on life span will result from feeding such diets. The present observations together with those noted in mice underfed at various ages (2) suggest that age differences exist in regard to the susceptibility to dietary changes. Further investigations will have to define the limits within which high fat diets are beneficial to longevity and will also have to supplement the present data in regard to the possible role of age in determining the effects of diets on life span.

Summary

Feeding a high fat diet from the ages of 6 or 12 months on shortened the life span of male mice of strain C57BL, but was less effective in this respect than feeding of this ration from one month of age on. Old mice were more susceptible to the adverse effect on life span than young adult animals. The effect of feeding the high fat diet for five months at a time depended on the period of life during which the fat-enriched diet was consumed. Given during the period of growth, the high fat ration did not alter the life span; in young adults it had a beneficial effect, while given to old animals it exerted a slightly injurious action on the life span.

Acknowledgments

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Sokoloff-'65
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Dietary Fat Supplements, Body Weight and Osteoarthritis in DBA/2JN Mice

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ABSTRACT Dietary supplements of saturated fats, either in the form of lard or hydrogenated safflower oil, had no demonstrable deleterious effect on the development of degenerative joint disease in male DBA/2JN mice. Although the lard resulted in greater obesity than did the cottonseed oil supplement, the weight gain was more rapid and greater when safflower oil was used rather than 2 hydrogenated products of safflower oil having iodine numbers equal to that of the cottonseed oil and lard, respectively.

A proposed osteoarthritis-enhancing action of dietary fat supplements has been the subject of a number of investigations. Silberberg and co-workers (1–4) reported that lard has a specific deleterious effect on the joint disease in mice. The action of lard was attributed to its non-specific arthritogenic influence in mice by virtue of its high caloric content and, in the case of the Y strain, through production of extreme obesity. Others reported, however, that a dietary regimen in which a vegetable shortening provided 87% of the metabolizable calories had no influence on the development of degenerative joint disease in 3 inbred strains of mice (5). Although this was a high fat ration, it had been diluted with an inert filler to the same caloric density as the stock chow. One possible explanation for the disparity between these observations in the articular changes was the type of fat used in the supplement — cottonseed oil was reported to be less injurious than lard (6). The following experiments were carried out to test this explanation.

METHODS AND MATERIALS

The animals used were male DBA/2JN mice. The experimental procedures, including evaluation of the joint disease by the papain-maceration method, were the same as those described in previous studies from this laboratory (5).

Experiment 1 was performed in 1961–62. The diets were the same as the three used by Silberberg and Silberberg (6). One was a control ground laboratory chow [2] (diet 10,000); the two others consisted of 74 parts of ground chow, 1 part of a commercial vitamin B mixture [3] and 25 parts of lard (diet 10,063) or cottonseed oil [4] (diet 10,062). Because of an impending high mortality in the lard-enriched group, the experiment was terminated when the mice were 14.5 months old, rather than 16 as originally planned.

Experiment 2 was conducted in 1962–63. Although the difference in the degree of unsaturation of the lard and cottonseed oil is considerable, there is little variation in the number of carbon atoms in their constituent fatty acids. To obviate any problems that might be associated with the slight differences in fatty acid chain lengths and the presence of trace substances that might influence the results, a highly unsaturated fat, safflower oil,[5] was chosen for this study (diet 10,080). One batch of the refined safflower oil, edible

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[2] Purina Laboratory Chow, Ralston Purina Company, St. Louis.

[3] This vitamin mixture contained in each gram: (in milligrams) inositol, 5; choline chloride, 75; menadione, 2.25; *p*-aminobenzoic acid, 5; niacin, 4.5; riboflavin, 1.0; pyridoxine·HCl, 1.0; thiamine·HCl, 1.0; Ca pantothenate, 3.0; biotin, 0.02; folic acid, 0.09; vitamin B_{12}, 0.0012; ascorbic acid, 45; α-tocopherol, 5; and vitamin A (as the acetate), 900 IU; and vitamin D, 100 IU (obtained from Nutritional Biochemicals Corporation, Cleveland). No odor of rancidity was ever apparent in any of our rations, and they were routinely checked for this point throughout the experiment.

[4] Wesson Oil, The Wesson Oil Company, New Orleans, Louisiana.

[5] Obtained from Drew Chemical Corporation, Boonton, New Jersey.

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grade, was hydrogenated to an iodine number of 110 (diet 10,081) which is the same as that of the cottonseed oil used in the first experiment (table 1). Another batch was hydrogenated to an iodine number of 55 (diet 10,082) which is the same as that of the lard (table 1). The orginal safflower oil and the hydrogenated fats were used in the diets at the same levels as in experiment 1.

The 3 safflower oils were analyzed for fatty acids by means of the gas chromatograph and found to have the composition listed in table 2.

TABLE 1

Fat supplements used in rations

Diet no.	Fat [1]	Iodine no. of fat
10,000	none	—
10,062	cottonseed oil	110
10,063	lard	55
10,080	safflower oil	145
10,081	hydrogenated safflower oil	110
10,082	hydrogenated safflower oil	55

[1] The fats were added at a level of 25% of the ration. The remainder consisted of 1% vitamin fortification mixture and 74% ground commercial laboratory chow (Purina Laboratory Chow, Ralston Purina Co., St. Louis).

TABLE 2

Fatty acid composition of dietary fats [1]

Fatty acid	No. of safflower oil		
	10,080	10,081	10,082
	%	%	%
16:0 [2]	5.8	6.4	6.3
18:0	1.7	3.0	30.9
18:1	11.7	51.5	62.8
18:2	80.8	39.1	0.0

[1] Values are percentages of total peak area.
[2] The first number represents the number of carbon atoms; the second the number of double bonds.

RESULTS

Experiment 1. Mice receiving the fat supplements became significantly heavier ($P < 0.01$) than the control animals, more so with lard than with cottonseed oil (fig. 1, table 3). Bone length, as measured in the femur, was not affected by the diets. The osteoarthritis scores of the mice fed the lard ration were close to those of the mice fed the stock ration; there was a questionable reduction ($0.05 < P > 0.01$) in the amount of joint disease in the mice

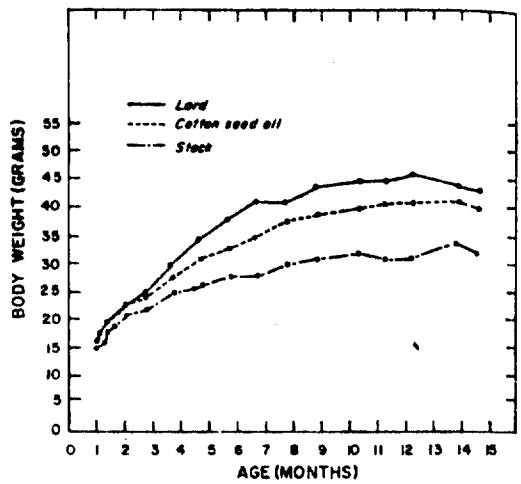

Fig. 1 Weight curves for rats fed lard, cottonseed oil or stock rations (exp. 1).

receiving the cottonseed oil-enriched ration when compared with the animals fed the basal stock ration (table 3).

Experiment 2. The mice fed the fat-supplemented rations attained significantly higher body weights than did the animals fed the basal stock diet (table 3). The weight gain was most rapid as well as greatest in the mice receiving the ration containing the unhydrogenated safflower oil (ration 10,080). The rate of increase and the maximal weights attained were less the greater the hydrogenation of the safflower oil (fig. 2). The femur lengths

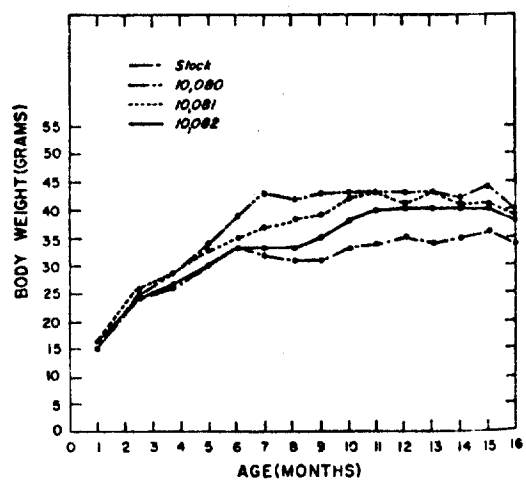

Fig. 2 Weight curves for rats fed stock ration, or safflower oil rations nos. 10,080—10,082 (exp. 2).

TABLE 3

Summary of observations (exps. 1 and 2)

	Diets, exp. 1			Diets, exp. 2			
	Stock	Lard	Cottonseed oil	Stock	No. 10,080	No. 10,081	No. 10,082
Peak body weight, g [1]	33.6 ±0.4 [2] (20) [3]	48.2 ±1.5 (20)	42.8 ±1.5 (20)	37.1 ±1.0 (22)	46.8 ±1.6 (26)	45.7 ±1.4 (27)	43.0 ±1.0 (25)
Final body weight, g	33.0 ±0.5 (20)	42.9 ±2.2 (20)	39.9 ±1.5 (20)	34.3 ±1.1 (22)	40.4 ±1.9 (26)	38.2 ±1.8 (27)	38.1 ±1.4 (25)
Epididymal pad weight, g	1.07 ±0.08 (20)	3.24 ±0.34 (20)	3.00 ±0.27 (20)	1.40 ±0.19 (13)	3.10 ±0.36 (17)	2.68 ±0.38 (14)	2.80 ±0.25 (20)
Femur length, mm	15.26 ±0.04 (20)	15.37 ±0.04 (20)	15.44 ±0.04 (20)	15.65 ±0.06 (22)	15.45 ±0.04 (25)	15.62 ±0.06 (27)	15.57 ±0.08 (25)
Osteoarthritis mean score [4]	0.41 (40)	0.48 (40)	0.21 (40)	0.45 (44)	0.46 (52)	0.48 (54)	0.26 (50)
Osteoarthritis ridit [5]	0.500 (40)	0.519 ±0.041 (40)	0.424 ±0.032 (40)	0.500 (44)	0.522 ±0.032 (52)	0.549 ±0.032 (54)	0.463 ±0.028 (50)
Survivors	24/24	20/26	23/26	23/30	23/30	28/40	28/40

[1] Computed from peak weight of each animal rather than peak month value as in figures 1 and 2.
[2] Mean ± SE.
[3] The numbers in parentheses represent the number of measurements tested.
[4] Based on a severity scale of 0 to 4 (5), recorded for each knee separately.
[5] Because the arthritis scores are discontinuous, the ridit method of Bross (9), as previously adapted (5), has been employed for statistical evaluation of the joint disease. The stock group has been chosen as the identifiable distribution in each experiment; no confidence limits are attached to it.

were comparable in all groups except for a slight reduction in the animals fed the safflower ration. There were no significant differences in the osteoarthritis scores (table 3).

DISCUSSION

Under the conditions of these experiments, no evidence was found that supplementation of the diet with lard or with saturated safflower oil potentiates the development of degenerative joint disease in DBA/2JN mice. This was true even though the mice fed the various high fat diets became obese The latter was evident in both the increased weight of the epididymal fat pad and the increased body weights with no change in skeletal size as indicated by bone lengths (table 3). The failure of obesity to have a deleterious effect on the joints of mice has been a consistent observation in numerous previous studies in this laboratory (5).

The lack of agreement between our previous observations that high fat diets per se do not have an arthritis-promoting effect in mice and the observations of others that they do have an effect has not been resolved by the present experiments. This difficulty exists even though we attempted to make the experimental design as uniform as possible. The only dietary variable in our last experiment was the degree of hydrogenation of the fat.

Other factors such as the strain of mice, variations in experimental design or genetic drift may be responsible for the differences in results secured in our laboratory and that of the Silberbergs. That the strain of mice has to be considered is evident from the observation of Silberberg and Silberberg (4) that lard supplements increased osteoarthritis in C57BL but had only a slight effect in DBA mice.

The suggested difference in experimental design stems from the presumption that the experiment in which the mice were fed the original vegetable fat was performed by others without simultaneous controls for the lard and stock diets. The arthritis scores for the mice fed the vegetable oil diets appear to have been compared with data from experiments published some years earlier (6). The weights of the mice at one month of age as shown by the weight curves in that report suggest that the animals were not of uniform body weights at the start of the study.

Changes may have occurred in the mice during the past 10 years which could help in explaining the differences in results secured in the 2 laboratories. This suggestion is supported by the low mean arthritis scores in our experiments for the mice fed the stock diet. The difference is apparent when the data from the present experiments are compared with those secured in previous years. The mice used in the present study as well as those in three other groups studied at the same time had significantly lower arthritis scores than those consistently observed in previous years in the DBA 2JN animals. All mice were obtained from the same source — the Animal Production Unit of the National Institutes of Health (5).

Presumably, the above changes resulted from some genetic drift. Degenerative joint disease in mice is a genetically influenced disorder in which the inheritance is governed by multiple genes whose over-all effect is recessive in nature. This fact makes it impractical to use non-inbred mice for investigating nutritional or other factors that may contribute to the lesion unless such factors are of overwhelming importance. The DBA/2JN mice have been used here because they have an intermediate susceptibility to the lesions. Furthermore, they are not subject to certain intercurrent diseases that have interfered with previous studies. Several C57BL strains of mice have been avoided because they are at times affected by a pruritic dermatitis (5, 8) that influences the development of osteoarthritis.

It is recognized that obesity can be produced in certain strains of mice by feeding them a high fat diet (5, 10). The fat used in most of these earlier studies was a hydrogenated vegetable shortening. Other work suggests that the nature of the fat has a marked influence on the degree of obesity attained by rats fed high fat diets. The fats that were solid at room temperature produced much greater weight gains than the liquid fats or oils (11).

The observations of this study agree with the latter report only in part. We found that the mice fed the lard diets gained weight more rapidly than did those fed the cottonseed oil diets. These results are partially explainable on the consistency of the diet — the one containing large amounts of cottonseed oil was oily and separated on standing. The layer of oil on the surface of the feed made it difficult for the animal to eat the diet. With solid fats, the consistency of the ration was maintained indefinitely making it much easier for the animals to eat the diet.

The better growth of the mice fed the safflower oil compared with that of the animals fed the same oil after hydrogenation (fig. 2) is difficult to explain. On the basis of our own experience and that of Barboriak et al. (11), we would have expected the opposite effect.

To explain this anomaly, we might suggest that there was an alteration in the palatability of the ration as a result of hydrogenating the oil. Hydrogenation also may have changed the physical characteristics of the oil. This may explain why ration 10,083 containing safflower oil with an iodine number of 55 had a dry consistency when compared with the ration containing the less saturated oil (10,081) or the lard diet (10,063). Suggestive confirmation for a change in the palatability of the ration comes from the observation that the feed cups of the mice fed the safflower oil ration had to be filled more frequently than those of the animals fed rations containing the hydrogenated oil.

Furthermore, there is a possibility that the metabolic utilization of the hydrogenated fats may have been altered as a result of saturating some of the double bonds (table 2). The metabolic change may have stemmed from an increased proportion of stearic acid in the fat or a change in the normal *cis, trans* isomerization, or both.

The differential growth effect of the safflower oil and its hydrogenated products deserves further study.

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THE BIOLOGICAL VALUE OF OILS AND FATS

I. GROWTH AND FOOD INTAKE ON FEEDING WITH NATURAL OILS AND FATS

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It has been shown by different investigators that the growth of young rats fed butter-containing diets is better than if the butter is replaced by certain vegetable oils or fats (Schantz et al. ['40], using maize, soybean, cottonseed and coconut; Harris and Rosenfeld ['40], who used coconut and olive oil; Boer ['41], who employed olive and rapeseed oils; Freeman and Ivy ['42], coconut; Parrish et al. ['46], maize; Deuel et al. ['48], rapeseed oil; Euler et al. ['48], rapeseed oil; Nieman et al. ['52], groundnut oil).

In contradiction to these results a number of workers report that no superior growth could be observed in rats fed butter-containing diets (Deuel et al. ['44; '49], maize, cottonseed, olive, groundnut and soybean oil; Henry et al. ['45], groundnut, maize, cottonseed and soybean oil; Zialcita and Mitchell ['45], maize oil; Lassen and Bacon ['49], olive and cottonseed oil).

In the present investigation the growth-action of a large number of fats and oils has been studied. Since previous experiments had shown that the growth depends on the fat-content in the diet (Hoagland and Snider ['40; '41], Boutwell et al. ['43], Forbes et al. ['46], Barki et al. ['50]), each fat was investigated at different dosage levels, varying from 10 to 73 Cal. %.

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EXPERIMENTAL

For each fat a series of 8 groups, each consisting of 8 to 12 male 21-day-old Wistar-strain rats, was used. Occasionally the number of groups was smaller than 8. All groups belonging to the same test fat series were started simultaneously, but each test fat was investigated at a different point of time.

Seven of the 8 groups received a diet in which the test fat was mixed in amounts of 10, 20, 30, 40, 50, 60 and 73 Cal. %, respectively. The 8th group served as a reference standard; its diet contained 20 Cal. % of summer butterfat. The composition of the diets used for the investigation of a given test fat is recorded in table 1.

TABLE 1

Composition of the diets

DIET	RICE	CASEIN	YEAST	SALTS	BUTTER FAT	TEST FAT	CALORIES PER GRAM
	gm	*gm*	*gm*	*gm*	*gm*	*gm*	
C20-Summer butterfat	157.6	34.5	28.5	8.5	21.5	...	3.99
C10-test fat	187.8	32.1	28.5	8.5	...	10.8	3.74
C20-test fat	157.6	34.5	28.5	8.5	...	21.5	3.99
C30-test fat	127.4	36.9	28.5	8.5	...	32.3	4.28
C40-test fat	97.2	39.4	28.5	8.5	...	43.0	4.62
C50-test fat	67.0	41.8	28.5	8.5	...	53.8	5.01
C60-test fat	36.8	44.2	28.5	8.5	...	64.5	5.48
C73-test fat	.	47.2	28.5	8.5	...	77.6	6.18

All of the diets contained the same amounts of protein, water-soluble vitamins (dried brewers' yeast), and salts per caloric unit; e.g. 230 Cal. protein, 28.5 gm yeast, and 8.5 gm salt-mixture per 1000 Cal. The content of test fat in the diets was varied at the expense of the carbohydrate content on a caloric base. Food and drinking water were given ad libitum; the diets were prepared twice a week. The fat-soluble vitamins were administered in the form of a prophylactic dose. At the beginning of the experiment and again after 4 weeks the animals were fed by pipette 0.05 ml of a concen-

trate containing 2000 I.U. vitamin A, 12.5 μg vitamin D_2, and 10 mg tocopherol-acetate, dissolved in groundnut oil. The animals were weighed weekly in the course of the 6-week experiment. The quantity of food supplied was also determined, but the wastage was not taken into account.

RESULTS

Twenty different oils and fats have been studied. Two of them, e.g. butter and whale oil, were investigated in duplicate. The results of the increase in weight after one, three and 6 weeks are shown in table 2.

The data obtained with the two summer-butterfat groups show that the rate of growth remains practically unchanged when the fat content of the diets is increased. Only in the first few weeks and at the highest dosage, viz. 73 Cal. % summer butterfat, is the growth somewhat less than at the lower dosages; but at the end of the experiment this difference has disappeared. With the other fats, however, with the exception of lard, there was, to a greater or lesser extent, a decrease of the rate of growth with increasing fat dosages.

In order to compare the growth-promoting effect of various fats with each other and in particular with summer butterfat, the difference between a test group and the simultaneously started standard group (20 Cal. % summer butterfat) was considered a suitable criterion. The comparison with butterfat was facilitated by deducting the mean value of the two butterfat series from the differences obtained. The growth-promoting value of summer butterfat was thus zero.

In table 3 a survey is given of the results obtained after statistical treatment of the experimental data. Only the observations after three weeks are recorded. The order in which the various fats are placed is determined by the magnitude of the differences as compared with summer butterfat. Since the growth of the rats in the butterfat groups after three weeks is about 100 gm, the numerical values in the table represent approximate percentages.

TABLE 2

Mean increase in weight after 1, 3 and 6 weeks (gm)

Cal. %	Lard				Summer Butterfat I				Summer Butterfat II			
	n	1 w	3 w	6 w	n	1 w	3 w	6 w	n	1 w	3 w	6 w
10	12	24	99	195	12	27	107	206	12	25	100	196
20	10	22	100	199								
30	12	25	109	216	12	26	109	218	12	25	104	211
40	12	25	107	211	12	27	108	211	12	25	105	206
50	12	25	105	218	12	25	111	220	12	24	105	218
60	12	24	104	220	12	26	110	211	12	24	103	217
73	12	23	105	220	12	21	103	216	12	21	99	208
Standard [1]	12	22	97	194	12	28	111	212	12	25	104	205

Cal. %	Olive Oil				Cottonseed Oil				Winter Butterfat			
	n	1 w	3 w	6 w	n	1 w	3 w	6 w	n	1 w	3 w	6 w
10					12	23	101	202	12	20	79	153
20	12	24	103	201	11	21	98	202	12	19	81	171
30					12	24	103	209	12	19	82	171
40	12	22	96	195	12	24	107	217	12	15	80	176
50					12	22	97	204	12	18	78	170
60	12	19	99	203	11	21	99	213	12	14	78	177
73	12	17	90	185	12	17	87	196	12	13	70	181
Standard [1]	12	24	102	195	12	24	101	209	12	18	80	166

Cal. %	Beef Fat				Shea Butter				Maize Oil			
	n	1 w	3 w	6 w	n	1 w	3 w	6 w	n	1 w	3 w	6 w
10	12	28	108	208	12	25	95	179	9	21	93	188
20	10	26	105	196	12	24	95	178	9	23	98	199
30	12	28	108	210	12	22	89	169	9	22	94	192
40	12	26	105	202	12	21	90	171	9	23	99	202
50	12	27	106	210	12	20	87	169	9	25	104	216
60	12	27	106	209	12	20	89	169	9	21	92	195
73	12	18	93	201	12	19	88	174	8	16	74	181
Standard [1]	9	28	111	213	12	22	93	181	10	22	94	189

Cal. %	Soyabean Oil				Groundnut Oil			
	n	1 w	3 w	6 w	n	1 w	3 w	6 w
10	11	26	102	190	10	25	107	214
20	11	26	100	184	10	24	104	215
30	11	26	103	198	10	25	109	219
40	11	24	92	184	9	22	102	210
50	11	24	95	188	10	22	105	215
60	11	23	97	200	10	21	99	210
73	11	17	82	184	10	14	84	193
Standard [1]	10	25	102	192	10	24	106	219

[1] Twenty Cal. % summer butterfat.

TABLE 2 *(continued)*

Mean increase in weight after 1, 3 and 6 weeks (gm)

Cal. %	Palm fat				Sunflowerseed oil				Poppyseed oil			
	n	1 w	3 w	6 w	n	1 w	3 w	6 w	n	1 w	3 w	6 w
10	9	27	106	204	11	26	102	204	11	24	98	197
20	9	28	104	203	12	26	103	208	11	24	98	199
30	9	30	110	207	11	25	101	208	12	24	101	207
40	9	29	110	212	12	24	103	207	11	24	99	202
50	8	27	107	214	12	24	101	214	11	24	101	207
60	8	23	100	197	10	21	88	191	11	20	89	200
73	9	16	91	193	12	17	75	177	11	13	69	177
Standard [1]	9	29	111	218	12	26	105	204	11	25	104	208

Cal. %	Coconut fat				Sesame oil				Owalanut oil			
	n	1 w	3 w	6 w	n	1 w	3 w	6 w	n	1 w	3 w	6 w
10	12	25	98	196	11	22	89	179	12	23	96	...
20	12	23	95	195	10	24	97	192	12	21	89	...
30	12	24	98	197	11	21	90	186	12	22	92	...
40	12	22	92	191	10	20	86	183	12	18	85	...
50	12	21	92	198	11	19	81	182	12	18	81	...
60	11	18	83	182	11	19	76	173	12	15	75	...
73	11	13	65	156	9	10	49	139	12	6	31	...
Standard [1]	12	26	102	203	11	22	91	190	12	22	94	

Cal. %	Whale oil I				Whale oil II				Herring oil			
	n	1 w	3 w	6 w	n	1 w	3 w	6 w	n	1 w	3 w	6 w
10	11	25	99	187	10	24	97	201	12	29	107	209
20	12	23	85	169	10	20	84	181	12	23	96	189
30	11	22	85	168	10	20	85	178	12	22	90	180
40	11	20	82	163	10	20	80	177	12	19	82	164
50	11	19	74	141	10	18	76	175	12	14	70	151
60	12	14	61	149	11	13	67	168	12	12	60	127
73	11	6	47	135	8	6	48	158	12	5	44	104
Standard [1]	11	23	97	186	10	21	100	214	12	29	108	212

Cal. %	Rapeseed oil				Kapokseed oil			
	n	1 w	3 w	6 w	n	1 w	3 w	6 w
10	8	21	93	183	12	16	88	187
20	10	22	81	154	12	15	73	169
30	9	19	75	149	11	12	57	142
40	9	13	56	131	12	7 animals died (mean 18 days)		
50	9	12	52	125	12	9 animals died (mean 15 days)		
60	9	7	35	96	12	all animals died (mean 14 days)		
73	9 (all animals died, mean 17 days)				11	all animals died (mean 13 days)		
Standard [1]	9	24	94	177	11	24	104	212

[1] Twenty Cal. % summer butterfat.

Table 3 shows that the difference between growth with summer butterfat and the other oils and fats increases with increasing fat content in the diet. Since the growth-promoting effect of summer butterfat is practically independent of the dosage, the augmentation of the difference is actually due to a lowering of the rate of growth caused by the different oils and fats when their dosage is increased.

TABLE 3

Difference in growth-promoting value (gm) as compared with summer butterfat (3rd week)

GROUP	TYPE OF FAT	CALORIC PERCENTAGE TEST FAT IN THE DIET						
		10	20	30	40	50	60	73
1	Lard	+ 5	+ 3	+ 12	+ 10	+ 7	+ 7	+ 14
2	Summer butterfat (Av.)	0	0	0	0	0	0	0
3	Olive oil	-	+ 1	-	— 6	-	— 4	— 6
	Cottonseed oil	+ 4	— 3	+ 3	+ 7	— 5	— 1	— 8
	Winter butterfat	0	— 1	0	— 2	— 5	— 4	— 6
	Beef fat	+ 3	— 3	+ 1	— 3	— 3	— 2	— 10
	Shea butter	+ 3	— 1	— 6	— 5	— 9	— 6	— 2
4	Maize oil	+ 2	+ 4	0	+ 5	+ 8	— 2	— 15
	Soybean oil	+ 5	0	+ 3	— 9	— 6	— 3	— 12
	Groundnut oil	+ 3	— 3	+ 2	— 6	— 4	— 8	— 18
	Palm fat	— 1	— 6	0	0	— 4	— 9	— 13
5	Sunflowerseed oil	0	— 2	— 4	— 2	— 5	— 17	— 24
	Poppyseed oil	— 2	— 5	— 2	— 4	— 4	— 14	— 29
	Coconut fat	0	— 5	— 2	— 9	— 9	— 17	— 30
6	Sesame oil	— 2	+ 2	— 4	— 8	— 15	— 18	— 50
	Owalanut oil	+ 5	— 5	— 2	— 9	— 14	— 19	— 58
7	Whale oil (Av.)	+ 3	— 14	— 13	— 17	— 24	— 35	— 45
	Herring oil	+ 1	— 12	— 19	— 27	— 40	— 48	— 59
	Rapeseed oil	— 3	— 18	— 24	— 44	— 49	— 64	†
8	Rapokseed oil	— 13	— 31	— 46	†	†	†	†

From a statistical analysis[1] of the figures it was found that, except for random sampling variations, the variability of the increase in weight after three weeks was the same for all groups. This quantity appears to be independent of the growth rate, the season, and the number in the litter. The best estimate for the variance of the increase in weight in the third week, derived from 1983 observations was found to be $c^2 = 118.1911$. Moreover the analysis showed that the distribution may be considered normal. The values in table 3 which differ significantly ($P = 0.05$) from those of the corresponding summer-butterfat group are boxed in.

In table 3 the various oils and fats are grouped according to the results of the significance test.

This classification of the different oils and fats is fairly artificial and is of value only for purpose of orientation. The fats of group 3, for example, do not differ significantly from summer butterfat, but they were grouped separately, since the differences with respect to summer butterfat at dosages of 50, 60, and 73 Cal. % all have a negative sign. Moreover, their total mean at a dosage of 73 Cal. % differs significantly from summer butterfat.

The further analysis of the gain data concerns the type of the growth curve. It has been found that the increase in weight when the standard diet with 20 Cal. % summer butterfat is administered is practically linear for the first 6 weeks. The growth index is $\frac{\text{increase in weight 3-6 weeks}}{\text{increase in weight 0-3 weeks}} = 0.985$ for a series of 37 standard groups.

The standard error of this mean $= 0.092$, so that the fiducial limits for $P = 0.05$ are : 0.789 and 1.151.

With the aid of these values it is possible to establish which growth indices deviate significantly from the normal standard (20 Cal. % summer butterfat). Table 4 records these growth indices. The values which deviate significantly are italicized.

[1] The statistical problems were studied in collaboration with the "Mathematisch Centrum," Amsterdam. In this connection I wish to thank especially Professor Dr. J. Hemelrijk and his co workers.

TABLE 4

Growth indices and efficiency constants

TYPE OF FAT	GROWTH INDICES $\left(\frac{\text{INCREASE IN WEIGHT 3–6 WEEKS}}{\text{INCREASE IN WEIGHT 0–3 WEEKS}}\right)$								EFFICIENCY CONSTANTS $\left(\frac{\text{INCREASE IN WEIGHT IN 6 WEEKS}}{\text{FOOD CALORIES IN 6 WEEKS}} \times 100\right)$								
	Standard (20 Cal. %) summer butterfat	Caloric percentage test fat in the diet							Standard (20 Cal. %) summer butterfat	Caloric percentage test fat in the diet							
		10	20	30	40	50	60	73		10	20	30	40	50	60	73	mean
Lard	1.09	0.97	0.99	0.98	0.97	1.08	1.12	1.10	7.13	7.84	7.65	7.56	6.71	7.46	7.07	6.90	7.29
Summer butterfat I	0.91	0.93	0.91	1.00	0.95	0.98	0.92	1.10	8.21	*9.80*	8.21	8.23	8.25	8.55	7.60	8.10	8.37
II	1.01	0.96	1.01	1.03	0.96	1.08	1.11	1.10	8.24	7.52	8.24	7.73	8.25	7.64	7.89	8.00	7.94
Olive oil	0.91	...	0.95		1.03	...	1.05	1.06	...	...	...	...	...	...	...	...	...
Cottonseed oil	1.05	1.00	1.06	1.03	1.03	1.10	1.15	*1.25*	8.14	8.00	7.68	8.94	8.53	8.80	8.29	8.32	8.34
Winter butterfat	1.08	0.94	1.11	1.09	*1.20*	*1.18*	*1.27*	*1.59*	6.29	6.69	6.33	6.90	6.40	7.15	6.91	8.15	6.85
Beef fat	0.92	0.93	0.87	0.94	0.92	0.98	0.97	*1.16*	7.54	8.36	7.13	*9.24*	7.71	7.24	6.89	7.40	7.69
Shea butter	0.95	0.88	0.87	0.90	0.90	0.94	0.90	0.98	6.40	7.10	*6.20*	6.32	*5.41*	*5.94*	*6.01*	7.15	6.32
Maize oil	1.01	1.02	1.03	1.04	1.04	1.08	1.12	*1.45*	7.94	8.70	8.52	8.54	8.93	9.09	8.83	8.52	8.64
Soybean oil	0.88	0.86	0.84	0.92	1.00	0.98	1.06	*1.24*	7.58	8.02	6.97	8.52	7.37	8.01	8.32	8.25	7.88
Groundnut oil	1.07	1.00	1.07	1.01	1.06	1.05	1.12	*1.30*	8.23	8.82	8.50	9.05	8.43	8.26	8.76	8.51	8.57
Palm fat	0.96	0.92	0.95	0.88	0.93	1.00	0.97	1.12	9.20	9.21	*9.25*	*9.42*	8.43	*9.53*	7.85	8.63	8.92
Sunflower-seed oil	0.94	1.00	1.02	1.06	1.01	1.12	*1.17*	*1.36*	8.13	7.39	8.43	8.74	*9.53*	7.50	7.95	8.17	8.21
Poppyseed oil	1.00	1.01	1.03	1.05	1.04	1.05	*1.25*	*1.57*	8.24	8.36	8.14	9.17	7.81	8.44	9.10	8.53	8.47
Coconut fat	0.99	1.00	1.05	1.01	1.08	1.15	*1.19*	*1.40*	8.31	8.20	8.04	8.83	7.50	8.14	7.81	8.07	8.11
Sesame oil	1.08	1.00	0.99	1.08	1.13	*1.24*	*1.27*	*1.84*	6.82	6.85	7.02	7.24	7.40	7.59	7.28	7.48	7.21
Owalanut oil	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Whale oil I	0.91	0.89	0.99	0.98	0.99	0.91	*1.44*	*1.87*	7.38	...	...	7.36	7.84	7.09	7.86	8.33	7.64
II	1.14	1.07	1.15	1.09	*1.21*	*1.30*	*1.51*	*2.29*	8.34	8.33	7.67	7.88	7.87	7.83	8.22		8.02
Herring oil	0.96	0.95	0.97	1.00	1.00	*1.16*	1.12	*1.36*	7.55	8.22	6.99	7.68	6.55	7.20	6.58	6.72	7.19
Rapeseed oil	0.88	0.97	0.90	0.99	*1.34*	*1.40*	*1.74*		7.30	7.48	6.45	6.82	7.37	7.72	6.91		7.15
Kapokseed oil	1.04	1.13	1.32	1.49					7.67	8.03	7.42	7.26					7.60
Mean	0.985 ± 0.092								7.73 ± 0.71	8.05	7.62	8.07	7.69	7.84	7.69	7.95	7.83

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Table 4 shows that a number of growth indices differ significantly from the standard, all these deviating values being too large. The data indicate that the deviation from the standard is greater as the rate of growth is smaller. The correlation between the increase in weight in the first three weeks and the magnitude of the growth indices appears to be highly significant ($r = -0.70$). Thus the type of the growth curve is generally linear (growth index $= \pm 1$), but in the case of certain oils and fats with little growth-promoting action, given in a large dose, the rate of growth in the second half of the experiment is better than in the first half. A certain "adaptation" manifests itself, which becomes more pronounced as the growth rate of the animals is diminished.

The data concerning the food intake refer to the total amount of food supplied in 6 weeks to each group. As already mentioned, the wastage of food, which will certainly have occurred and to a varying extent for the different groups, is not taken into account. As was to be expected, there is a positive correlation between the food intake and the increase in weight. For this reason the efficiency constants were calculated. Efficiency constant $= \frac{\text{increase in weight in 6 weeks}}{\text{food calories in 6 weeks}} \times 100$. The second part of table 4 records these efficiency constants. The average of the efficiency constants of the standard groups (20 Cal. % summer butterfat) is 7.73 with S.D. = 0.71. Values which differ significantly from this standard mean are in italics. With the exception of shea butter (too low) and palm oil (too high) the table suggests that the efficiency constants are approximately the same for all the fats and oils. It seems therefore that the increase in weight per calorie of food intake is independent of the type of fat and the level at which it is administered.

Finally, some data are given on the mortality observed in some of the experimental groups. Of the group which received the diet with 73 Cal. % of rapeseed oil all animals died, after 8, 10, 12, 14, 15, 20, 20 and 37 days, respectively (mean 17 days).

The mortality in the kapokseed oil groups was as follows:
40 Cal. % : 7 of the 11 animals died after 6, 14, 18, 18, 21, 21 and 27 days, respectively (mean 18 days).
50 Cal. % : 9 of the 12 animals died after 11, 11, 12, 15, 16, 16, 17, 19 and 20 days, respectively (mean 15 days).
60 Cal. % : all animals died after 8, 10, 13, 14, 15, 15, 16, 16, 17, 18, 19 and 21 days, respectively (mean 14 days).
73 Cal. % : all animals died after 8, 9, 11, 12, 12, 12, 13, 14, 15, 15 and 20 days, respectively (mean 13 days).

DISCUSSION

The 20 oils and fats investigated do not all have the same effect on growth, but show certain differences. It is illogical to explain this by the theory of the presence of an unknown "growth-promoting" substance in butter. For in that case the favourable substance should not only occur in butter but also in the other oils and fats, the content diminishing with decreasing growth-promoting effect. As a consequence, on raising the dosage with these oils and fats an improvement of the growth should be expected, while, on the contrary, a decline has been observed. Moreover, the "deficiency," which will develop by depletion of the growth-promoting substance, starts at the beginning of the experiment so that a so-called "run-out time" is missing. Besides, the values of the growth indices of the oils and fats with low growth-promoting effect are higher than 1, which points to a certain improvement where an aggravation could be expected. All these facts argue against the growth-promoting hypothesis.

In contrast to this, a growth-retardation theory in which the presence of "growth-retarding" substances in various oils and fats is assumed, explains both the lowering of the rate of growth on raising the fat content in the diet as well as the growth differences obtained with the various oils and fats investigated. The poorest-acting oils and fats should contain the largest amounts of retarding substance(s) and the "retarding potency" should decrease as the growth-action of the fat improves. This theory has received support in the

finding that erucic acid, which is present to the extent of about 50% in rapeseed oil, was responsible for the poor growth-action of this oil (Thomasson and Boldingh, '55).

Nothing is known about the nature of any growth-retarding substance in the other poorly-acting oils and fats. Assuming them to be fatty acids, attention is focused on the long-chain fatty acids with 20 or more carbon atoms. Leaving the degree of unsaturation out of account, owalanut oil contains 23% of 20-carbon atom acid, whale oil and herring oil 20 and 52% respectively of 20- and 22-carbon atom acids, and rapeseed oil 50% of 22-carbon atom acid. On this basis the hypothesis is suggested that fatty acids with 20 or more carbon atoms have an unfavourable influence on the growth of young animals. Unfortunately, the composition of kapokseed oil is not well-known; otherwise further suggestions could possibly be obtained from this quarter.

The results indicate that the various oils and fats, with the exception of shea butter and palm oil, seem to have the same food efficiency in spite of differing values in promoting growth. Therefore, it is probable that irrespective of the kind of fat and the level at which it is administered, the caloric value for the animal is the same; and moreover that the growth-retarding fatty acid will be metabolized to the same extent and in the same way as the non-retarding ones, but that only the *rate* at which metabolism takes place is different for the various fatty acids. The low caloric value of shea butter may be a result of the high content of unsaponifiable matter (5 to 7%). The cause of the high value of the efficiency constant of palm oil is not known.

The fact that the food intake of the rat can be regulated by means of the type of fat opens up the possibility of a practical application in medicine, insofar as this rule also holds good for man. In particular, a fat which lowers the food intake will have a chance in the treatment of those forms of obesity which are due to excessive food intake. This problem is under further investigation.

The growth and food intake of the animal form a generally accepted criterion for judging its state of health and a retarded rate of growth is considered as an expression of a pathological condition, or a dysfunction. On this basis the biological value of the oils and fats with a good growth-promoting effect will be rated higher than those with a poor action. This view seems to be supported by the fact that the animals on diets containing high dosages of rapeseed oil or kapokseed oil died within the 6-week experimental period. This conception, however, is incorrect, as has become apparent from a further investigation (Thomasson, '55). It appeared that rats fed 50 Cal. % of rapeseed oil lived significantly longer than rats on a diet containing 50% summer butterfat, although the growth rate, and the daily food intake, of the rapeseed oil animals were much poorer than those of the animals fed butterfat. This observation indicates that growth-inhibiting substances in oils and fats need not of necessity have an adverse effect, but can have a favourable influence instead. In addition, the longer life span observed supports the conception that growth-retarding substances can be useful in the therapy of obesity. Finally, this investigation argues strongly against the existence of an unknown vitamin in butter. For, now that it has appeared that animals receiving this factor — the butter group — have a shorter life span than the "deficient" rapeseed oil animals, the existence of such an unknown butter factor becomes highly dubious.

SUMMARY

In 6-week feeding experiments with rats the behaviour of 20 oils and fats has been investigated, each of them being administered in 7 different dosages from 10 to a maximum of 73 Cal. %.

The various oils and fats showed differences in growth-promoting effect. Nevertheless, the food-efficiency seemed to have a constant value and to be independent of the type of fat used. Only shea butter and palm oil were exceptions to this rule.

As an explanation of the differences observed the presence of growth-inhibiting substances is suggested. Such substances need not be considered as harmful, but may possibly even exert a favourable influence, judging from the data on longevity.

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INFLUENCE ON THE CHILDREN OF A MATERNAL DIET TOO RICH IN FAT
[Le ripercussioni sulla prole di un'alimentazione materna troppo ricca di grassi]

by

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INFLUENCE ON THE CHILDREN OF A MATERNAL DIET TOO RICH IN FAT

Report I.

RESEARCH ON RATS GIVEN AN EXCESSIVELY HIGH PORK FAT DIET DURING PREGNANCY AND LACTATION

S. Santyan Y Velasco and G. Pancaldi

The frequent finding in newborn and suckling babies seen in the out-patient department of our clinic of a considerable increase in the size of the liver and the tendency for disturbances associated with functional hepatic insufficiency – spontaneously or during various diseases – has caused us to become particularly concerned about the problem.

It is likely that neuroarthritic heredity plays a role here and that a congenital familial hepatism is therefore involved. But we were concerned with the problem as to whether the use of a heavy fat diet in this area may not be of such an importance as to require investigation.

We also observed in many babies the frequency of exudative diathesis with the various cutaneous and general manifestations linked to this problem, and we know from Czerny that it is precisely the fat component which is capable of arousing the latent diathetic state, provoking manifestations of disease. It is known how the abundant excesses of animal fats may produce eczematous lesions. The bonds between exudative and neuroarthritic diathesis are so close that their manifestations frequently overlap (phenomena of hepatism, hepatic and renal calculous conditions, seborrhea, intertrigo, eczema, gout, asthma, obesity, etc.). In fact, the exudative diathesis of the suckling and small baby would continue in the neuroarthritic diathesis of the larger baby and of the adult (Francioni and Pacchioni).

Before describing the results and the purpose of the investigations which are the subject of the present report, we feel that it is necessary to briefly recall the way in which fats are metabolized according to the most recent data.

It is commonly known that fats in the diet consist of neutral fats represented by esters of fatty acids with glycerin. In these fats the glycerin is combined with three acid radicals and we are thus concerned with triglycerides. The most common triglycerides, and therefore those which are found in the subcutaneous tissue and in other deposits, are mixtures of tripalmitin, tristearin, and triolein. We also find in the fats mixed glycerides, in which the alcohol groups of the glycerin are combined with two or three different radicals of fatty acids.

As we know, a certain amount of fats is almost indispensable in the diet, first of all because these fats constitute a valuable reserve material and secondly because they are the foods capable of displaying the greatest caloric value with the least volume (9.3 calories per g). Furthermore, some fats are vectors of accessory substances or vitamins, which have a very important effect on the body. According to Starling, fatty substances should be responsible for 30-35% and more of the daily calories counted in babies.

The fat is absorbed by the digestive tract, especially as alkaline saponin and glycerin and the neutral fat is resynthesized in the intestinal walls, reaching the blood by means of the chyligerous vessels. Some of this neutral fat already circulating in the blood is split by circulating lipases and the resulting fatty acid helps form complex molecular compounds, such as lecithin – desaturated and oxidized anew. Meanwhile another part of the fat, having reached the deposits, is divided again and then resynthesized by lipases, which are very abundant especially in the tissues of major importance for the metabolism of the fats. When the deposited fats must be removed, it is once again the lipases which split them and resynthesize them, until the neutral fat arriving in the consumption areas is finally transformed into fatty acid and glycerin. The glycerin is burnt and may produce sugar, which will be deposited in the form of glycogen. However, the fate of the fatty acids is

more complex. These mostly appear in the saturated series ($CnH_{2n}O_2$) and some appear in the unsaturated series (CnH_{2n}-2 O_2). The difference between the saturated and the unsaturated fatty acids lies in the fact that in the latter, there are double bonds in the open chain of carbon atoms due to the lack of hydrogen atoms. These double bonds may open and fix hydrogen or other elements capable of replacing it, such as the halogens.

In the intermediate exchange, we see in particular the formation of fatty acids with an equal number of carbon atoms. The most widespread of these are palmitic and stearic acid, which are also the principal constituents in animal fats. However, the unsaturated acids are also of great biological importance, the main one being oleic acid. There are also fatty acids with many double bonds, which are therefore less saturated than oleic acid, such as linoleic and linolic acid from linseed oil. According to the known theory of Knoop and Dakin, the saturated fatty acids are attacked by the unilateral beta-oxidation phenomenon, which is probably initiated in the liver (Leathes, Meyer, Sinclair) with the successive falling of two out of two carbon atoms. The separation of the two carbon atoms each time thus takes place by oxidation corresponding to the beta-carbon, or the second carbon atom in the chain from the carboxyl. F.P. Mazza, Verkade, and other authors also studied omega-oxidation and succeeded in demonstrating that, beside the classical process of degradation by unilateral beta-oxidation, the saturated fatty acids are definitely also broken down "by beta-oxidation in the omega position, subsequently followed by bilateral beta-oxidation of the dioic acids formed." And the unsaturated fatty acids? Their catabolism is not yet well known. Leathes and Raper support the theory that these are split by oxidation, due to their weak bond. Each fragment would then be broken down on its own account. On the other hand, Verkade thinks that beta- and omega-oxidation also play a large role in this process, based on what is known about the saturated acids. A few years ago Knoop, Oppenheiner, and Wieland acknowledged that succinic acid may play an important role in the

intermediary metabolism of the fatty acids, but it was not known whether this acid – which is also diffused throughout the muscles and the liver, etc. – comes from the fatty acids themselves, from the carbohydrates, or from the amino acids. The theory of Verkade on the catabolism of saturated fatty acids by omega-oxidation, followed by bilateral beta-oxidation, would permit us to consider the saturated fatty acids of alimentary fats as "very probable, if not certain, mother substances of succinic acid".

The liver is also extremely important in the digestion of fats, both as a bile-producing gland – which assists in the splitting of fatty acids and cholesterol by means of the bile salts – and as a storage place for these alimentary substances, as well as for proteins and especially carbohydrates. We have already mentioned that the first phase in the oxidation of fatty acids would be desaturation and that this process would take place in the liver. The storage and consumption of fats, as well as that of carbohydrates, is influenced by the hormonal secretions (thyroid, hypophyseal, genital) and by the effect of vitamins. Frank in 1923, Frontali in 1924, and Careddi in 1928 demonstrated that the B complex, and more precisely vitamin B_2 – which is water-soluble and thermolabile – promotes the use and tolerance of alimentary fats in young organisms and has an elective effect on development.

After these brief general explanations, we will consider more particularly the characteristics of the principal fats and oils that may commonly be used in the diet. For greater clarity, we have assembled the data concerning the principal fats and oils in a table. As for the chemical constitution of pork fat, which we used for this first series of investigations, we present more detailed data kindly provided by the Royal Institute of Biological Chemistry in Naples, directed by Prof. Quagliarello, to whom we express our gratitude.

The average values are obtained by analyses made on the perirenal fat, on the fatty layer, etc.

The fatty tissue of pork contains: 6.44% water; 1.35% connective tissue; 92.21% fat. In the composition of the fatty tissue of the pig, we thus see that unlike other fatty tissues (of the steer for example), there is a fairly high percentage of fat. The fat taken from the adipose tissue has the following composition: 76.54% carbon, 11.94% hydrogen, 11.52% oxygen, melting point 42-48°. It is essentially composed of triglycerides, oleic, stearic, and palmitic acid; linoleic acid is also present in small quantities. The solid triglycerides are represented according to A. Bömer by alpha-palmitodistearin (melting point 68.5) and stearodipalmitin (melting point 58.2). According to Amberger, the solid triglycerides are represented by beta-palmitodistearin. Tristearin does not seem to be present. We also report the percentages of the various triglycerides in the lard according to Bömer and Amberger:

beta-palmito-alpha. alpha-distearin	approximately	3%
beta-stearo-alpha. alpha-dipalmitin	"	2%
alpha-oleo-alpha. beta-distearin	"	2%
alpha-palmito-alpha. beta-diolein	"	82%
beta-oleo-alpha palmito-alpha-stearin	"	11%
Cholesterol:		0.2-0.3%

Contents of fatty acids in percent:

oleic acid	50-60%
palmitic acid	24.6-32.8%
linoleic acid	10%
stearic acid	7.8% up to 15.4%
Saponification index	195°
Iodine number	76.5

We began our research on May 30, 1938 with the breeding of 5 young female rats (at the start of the experiments, these rats were about two months old), all from a single litter. Two of these rats were fed bread, milk, and water at will. On the contrary for the other group of three rats, we added an overall daily quantity of 4-5 g of fat to the diet of bread, milk, and water. We also fed large quantities of fat to another female rat, already an adult, kept under the same conditions as the others. When we initiated our experiments, the first two rats, i.e. from the control lot, weighed 53 g and 54 g respectively. The others

Specific weight at 15°	Melting point of fatty acids	Saponification No.	Iodine No.	No. of fixed acids	No. of volatile acids
Olive oil 0.913-0.919	22-28	185-196	79-88	95-96	0.3
Groundnut oil 0.916-0.920	27-33	185-197	83-103	95-96	1
Rape oil and colza oil 0.913-0.918	17-24	167-180	94-106	95-96	0.5
Linseed oil 0.930-0.937	17-24	187-195	164-194	95-96	–
Cottonseed oil 0.922-0.931	34.40	190-198	101-117	95-96	–
Butter (at 100°) 0.865-0.868	38-45	220-232	26-40	85-91	25-30
Oleomargarine 0.924-0.930	40-43	192-200	42-55	95-96	0.4-1
Beef fat (suet) 0.943-0.953	40-49	193-202	36-56	95-96	0.5
Pork fat (lard) 0.931-0.938	36-48	195-200	46-70	95-96	–
Sheep fat 0.937-0.961	44-52	192-197	32-46	95-96	–

Table according to the data from the Treatise of Analytical Chemistry of Villavecchia.

weighed 51 g, 55 g, and 58 g. All were already on their way towards a rapid growth development. The adult female rat weighed 130 g at the beginning of the experiment. A single male weighing 105 g was alternately kept for one week with the two control rats and for one week with the rats on the diet supplemented with pork fat.

Our first observation was that a few days after initiation of the diet containing fat, the three rats who had previously eaten eagerly, began to lose their appetite, to become emaciated, and to crouch in a corner of their cage. Furthermore, their snout was somewhat swollen, the eyelids rather edematous and reddened with thinner eyelashes, rumpled fur and – a characteristic fact – the external genital organs were extensively reddened and swollen. The one male rat on an alternating diet, after three or four days with these rats, displayed the same disturbances, with a conspicuous swelling of the scrotum and of the testicles and priapism. These disturbances diminished until they disappeared after 9-10 days, to reoccur periodically and in a progressively more attenuated form in the female rats. The drive of the female rats, as well as their reproductive activity, and also the sexual activity of the male, were not in any way altered as may be concluded from the fact that one of the females gave birth to a litter, almost at the same time as one of the females in the group on a normal diet.

Some of the disturbances which we observed have already been described in rats by Frank and Frontali, and were attributed by the first author to an A hyperavitaminosis and by the second author to a partial B avitaminosis, since the disturbances were observed both in rats fed with fats rich in vitamin A as well as in rats fed with fats lacking this factor (soybean oil). However recently, W. Weslaw-Wronski, A. Wroblewski, and B. Wroblewski described a complex of symptoms completely identical to those partially observed by us, also accompanied by skeletal alterations (brittleness, softening of the bone) demonstrated radiographically, in rats subjected to A hyperavitaminosis either through the intestinal route, or subcutaneously and percutaneously. Our experiments were not concerned with making direct observations on the adult animals. We therefore limited ourselves to hastily mentioning this series of disturbances, without however trying to give an interpretation.

Our objective, as is indicated by the title of the paper, was to investigate the possible influences on the offspring of a diet overly rich in fats. We therefore immediately began to examine the observations which we made on the litters from the female rats in the experiment. From the chronological point of view, we note that approximately two months after initiation of the experiment (30 May), a rat on the normal diet gave birth to a litter on July 26 and a rat on the fat-supplemented diet gave birth to a litter on July 17. Then the second control rat gave birth on August 25, the second rat on the fat diet gave birth on September 3 and the third rat on the same on September 8, and finally the adult rat gave birth to a litter.

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First control litter (delivery 26 July). – Six healthy newborn rats of normal dimensions, quite animated in their movements, of a pink color. The mother and the newborns killed each other. Individual weights of the newborns: 5.87 g, 5.88 g, 5.12 g, 5 g, 5.94 g, and 5 g. Average weight: 5.47 g. Following delivery, the rat weighed 130 g (from 54 g). The liver of the rat weighed 5.87 g and was normal in coloring and consistency. The livers of the newborns, also normal in appearance, individually weighed: 28 cg, 28 cg, 27 cg, 28.8 cg, 30 cg, and 22 cg; the average weight was 27.3 cg. The histological examination on the liver of the rat and on the liver of her newborn babies, fixed in formaldehyde and then stained with Sudan III, did not display an abnormal presence of fat. The hepatic cells only displayed a few scanty drops of fat, which is a completely normal finding.

Second control litter (delivery 25 August). – Six newborns in a good general and nutritional state. The mother and the newborns killed each other. The first weighed 135 g (from 53 g) and each newborn weighed 6 g, 5.90 g, 6 g, 5.87 g, 5.95 g, and 6 g; the average weight was 5.96 g. The liver of the rat having a normal color and consistency weighed 5.90 g. The livers of her

newborn babies, also normal, weighed respectively 32 cg, 28 cg, 29 cg, 28 cg, 30 cg, and 30 cg; the average weight was 29.5 cg. The histological examination did not show any abnormal factors.

IInd*LOT*

First litter of newborns from the mother on the fat-supplemented diet (delivery 27 July). – Five baby rats were born, which were rather sluggish and fairly cyanotic in color. The mother and the newborns killed each other. The mother weighed 90 g (from 51 g) and the babies weighed respectively 4.80, 5.60, 4.70, 4.80, and 4.60 g; the average weight was 4.90 g. The liver of the mother, weighing 5.87 g, was fairly voluminous, rather yellowish in color, with a tendency here and there towards an ashen appearance, and somewhat diminished in consistency – all characteristics which also appeared on the surface of the incision. The livers of the newborns were dirty yellow or greyish in color and of a soft consistency, easily reduced to a pulp, and weighing respectively, 20, 20, 19.8, 19.5, and 19.8 cg; the average weight was 19.8 cg. In the perirenal areas of the rats, as well as along the mesentery and to a lesser extent in the subcutaneous tissue as well, there was an abundant deposit of fat having a mucilaginous appearance and a whitish color, similar to the fat that the rat had consumed. Both in the liver of the mother as well as in the liver of the newborns, the histological examination showed a more or less pronounced state of adiposis. The hepatic cells appeared to be strewn – especially at the periphery of the protoplasm – with numerous granules and droplets of fat varying in size, accumulated here and there in such a way as to form true and proper vesicles. The nucleus of some cells appeared to have moved to the periphery of the same cell and frequently the protoplasm was reduced to a thin edge.

Second litter of newborns from a mother on the fat-supplemented diet (delivery 3 September). – There were eight babies, which were much smaller in

size than the others, of sickly appearance, quite sluggish, intensely cyanotic, and with a parched, wrinkled skin. The mother and the newborns killed each other. The mother weighed 100 g (from 58 g). Each baby weighed respectively 3.60, 3.60, 3.40, 3.50, 3.20, 3.40, 3.50, and 3.40 g; the average weight was 3.40 g. The liver of the mother weighed 6.40 g and was voluminous, of a uniform yellowish tint, rather soft to the touch, and the incision surface appeared to be fairly moist, non-granulous, and of a dirty yellowish-red color. The liver of the newborns were small, yellowish-pink in color rather than pink as in the controls, soft in consistency, mushy, and weighing respectively 15, 19, 12, 19, 15, 12, and 19 cg; the average weight was approximately 16 cg. No aceni were observed at the surface of the incision, but this surface appeared compact and turbid. Upon the histological examination, we observed the abnormal presence of fat both in the liver of the mother as well as in the liver of the newborns. Conspicuous quantities of whitish, mucilaginous fat were also observed in the pararenal regions, along the mesentery, and in the subcutaneous tissue of this female rat.

Third litter of newborns from a mother on the fat-supplemented diet (delivery 8 September). – There were six babies, also in poor nutritional condition, with a cyanotic, wrinkled, and dry skin. The mothers and the newborns killed each other. The mother weighed 98 g (from 55 g). Each baby weighed respectively 3.80, 3.80, 3.70, 3.60, 3.40, and 3.50 g; the average weight was 3.6 g. The liver of the mother weighed 6.20 g and displayed the characteristics already described with respect to the rat mentioned above. The liver of the newborns was small, yellowish, lacking in consistency, and weighing respectively 17, 16, 18, 19.3, 17.9, and 15 cg (average weight 17.3 cg). An accentuated state of adiposis was observed both in the liver of the mother as well as in the liver of the newborns upon histological examination.

Fourth litter of newborns from a mother on the fat-supplemented diet (delivery 10 September). – The rat which weighed 130 g at the beginning displayed

the same disturbances and the same alterations as the preceding rats during pregnancy. The ten babies were in a poor state of nutrition, and were very cyanotic in color. The mother and the newborns killed each other. The mother weighed 150 g. Her liver weighed 8 g, was voluminous, yellowish-red in color, uniform in tint, little consistent upon palpation, and soft at the incision – the surfaces of which appeared to be fairly moist, turbid, and dull. There were acini. The newborns weighed 4.30, 4.20, 4, 5, 4.20, 5, 4, 4.70, 4, and 4.20 g. The average weight was 4.25 g. Their livers were blackish-red in color and were soft, capable of being reduced to a pulp, and weighing respectively 23, 23, 23, 22, 24, 22, 23, 22, 24, and 22 cg; the average weight was 23 cg. The histological examination of the liver in both the mother and in the newborns showed an accentuated state of adiposis.

As we already mentioned, we began our experiments with the breeding of two lots of pregnant rats. Each of these groups, except for the last one, was still in the rapid growth period since from a weight of 51-58 g, there was an increase after delivery to 90-135 g – almost double or more the initial weight. We already described the alterations present in the rat kept on a high-fat diet. Their growth was also fairly stunted. While the rats on the normal diet weighed 130-135 g after delivery, the experimental rats only reached the respective weights of 90-100-98 g. Obviously the fat-rich diet, provoking the phenomena described above, had harmfully influenced the growth of the animals.

The livers of the control rats – normal in appearance, size, color, and consistency, as well as in their histological structure – weighed 5.87 and 5.90 g (weight of the animals 130-135 g), while the livers of the rats on the fat-rich diet were voluminous, of a uniformly yellowish color, diminished in consistency, and with the incision surface fairly moist, shining, and oily. No acini were observed on this surface. The weight of the livers was 5.87, 6.40, 6.20, and 8g(weight of the rats 90, 100, 98, 150 g) – thus more disproportionate to the weight of the animals the longer they were fed the fat diet.

The histological examination carried out in order to determine the possibility of an abnormal presence of fat in the hepatic cells (pieces fixed in formaldehyde and then stained with Sudan III) always showed a more or less accentuated state of adiposis (Figure 1).

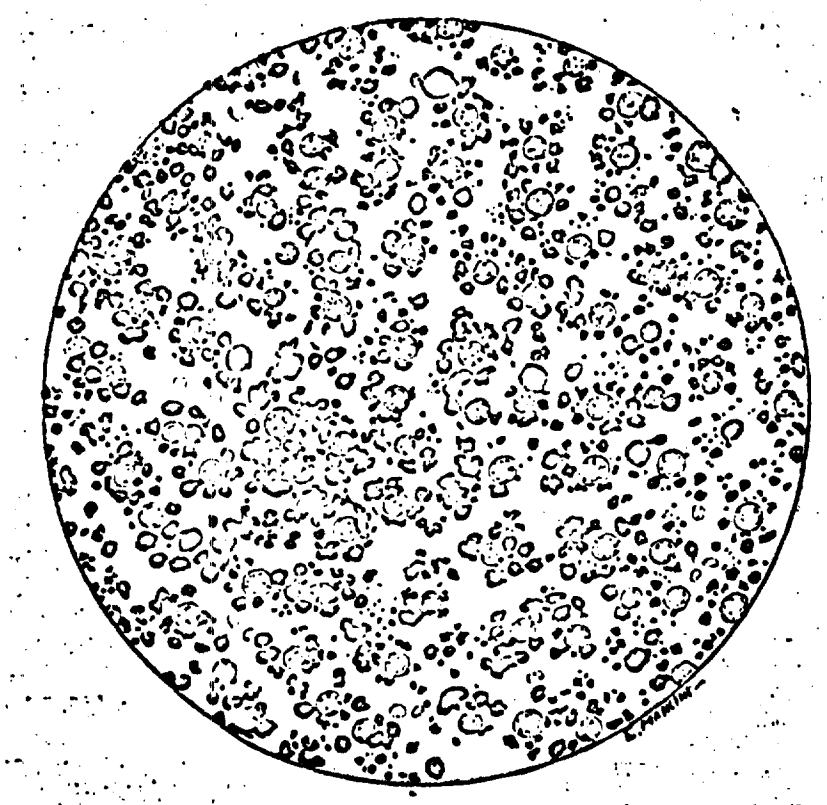

Fig. 1.

The adiposis of the liver of infiltrative origin – as is most probable (Lebedeft, Rosenfeld, Cavazza, etc.) – or of degenerative origin (Mavrakis, Wirchow, etc.) is in general "proportional to the richness of the fat deposit in the animal", according to Rondoni. We have seen that in the subcutaneous tissue, especially in the renal areas and along the mesentery of the rats on the special diet, a considerable quantity of fat having a characteristic appearance and color was present. This actually consisted of an alimentary fat deposit which can be stored directly by the organism, according to the experiments of Pettenkofer and Voit and, even better, those of J. Munk and Hoffmann. In fact,

while many authors only considered the endogenous origin of the fat deposited because it was thought that the alimentary fat was completely burned, the above-mentioned authors showed, on the contrary, by administering a large amount of fat and small quantities of protein to dogs which had previously been fasting – so as to deplete their fat reserves – that it is also possible for the fats introduced with food to be stored in the adipose deposits. According to Zummo, the external temperature would have a great deal of influence on the conposition of the type of fat deposited.

No difference is observed with respect to the number of newborns and the time of birth in the two groups of rats. On the contrary, with respect to the general and hepatic conditions and the nutritional state of these same newborns, a few words must be added. The two control rats each delivered six babies, lively in their movements, in generally excellent condition, well nourished, not displaying any skin alterations, and pink in color, which easily attacked their mother's breast, as demonstrated by the fact that their stomachs were full of milk one day after birth, at which time they killed each other. Their weight ranged from a minimum of 5 g to a maximum of 6 g, with an average of 5.71 g. The livers of these newborns were pink in color and quite consistent, with a weight ranging between a minimum of 22 cg and a maximum of 30 cg (average weight 28.4 cg). The histological examination showed whole hepatic cells or cells containing in their protoplasm sparse granules of fat, which represented a completely normal distribution (Figure 2).

The newborns of the mothers on the diet rich in pork fat, especially those of the rats on the special diet for a long time (more than three months), appeared weakened, sluggish in their movements, poorly nourished and rather sickly, sucking with difficulty, and of an intensely cyanotic color with a dry and wrinkled skin. Their weight ranged between 3.20 g and 5.60 g (only one reached the weight of 5.60 g), with an average weight of 4.04 g. The newborns

from the first and the fourth litter were in better condition than those in the other two. In fact, the mothers of the first and fourth litters had eaten pork fat for only approximately two months, while the mothers of the second and third litters had been eating pork fat since the beginning of the experiment up to the time of delivery, or for more than three months.

The liver of these newborns generally appeared small, yellowish in color, soft in consistency, non-compact, but easily reduced to a pulp, and ranging in weight between 12 cg and 24 cg, with an average weight of 19 cg – therefore noticeably less than that of the liver in the controls. The histological examination of the pieces fixed in formaldehyde and subsequently stained with Sudan III demonstrated a more or less pronounced state of adiposis (Figure 3).

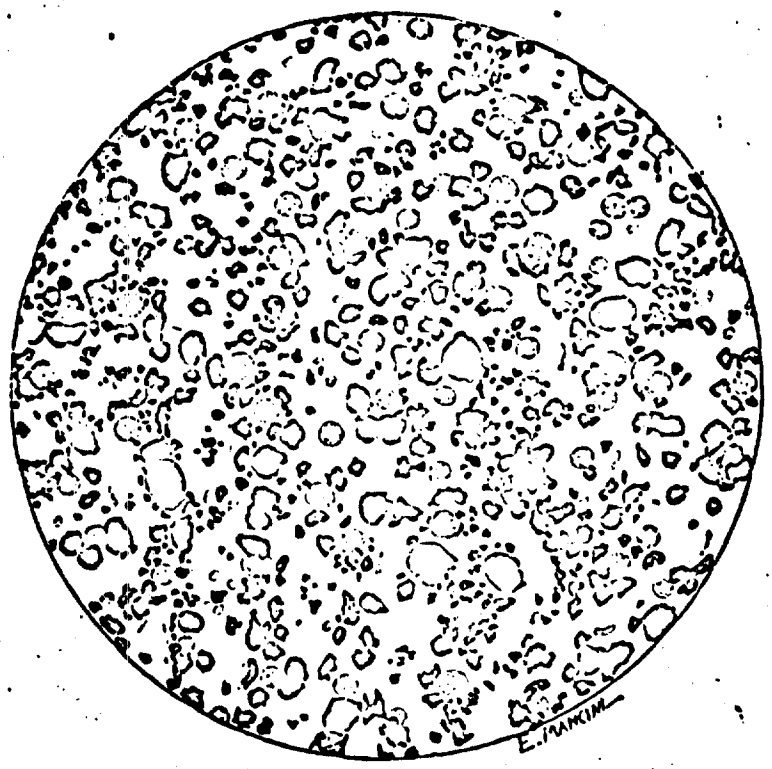

Fig. 2.

The hepatic cells appeared to be strewn with granules and drops of fat, especially abundant at the periphery of the cytoplasm and in some places grouped so as to form true and proper adipose vesicles. The nucleus of the same cells

frequently appeared to have moved to the periphery and the protoplasm appeared to be reduced.

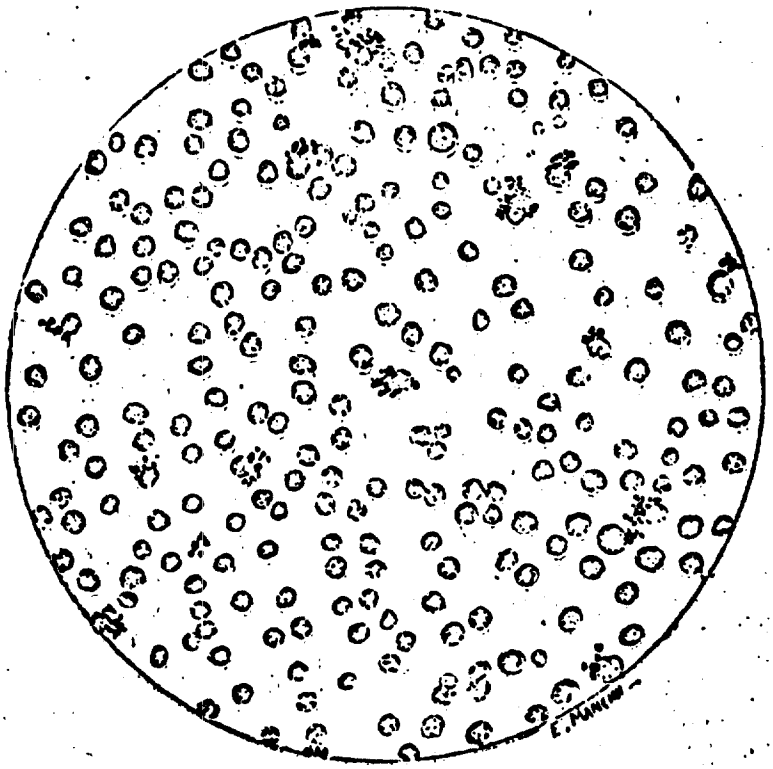

Fig. 3.

Leaving aside the incidental observations and considering only that of most interest to us, i.e. the difference existing with respect to the general and hepatic conditions, in addition to possible diathetic alterations or manifestations differing between rats fed a normal diet and rats fed a high-fat diet (pork fat), it is difficult to say that this has been distinct and clear for certain aspects. The rats kept on a diet rich in pork fat, in addition to displaying particular and varied disturbances and having a rather stunted growth, gave birth to babies in a poor state of general health and nutrition, weakened, with a parched skin, and displaying a more or less pronounced state of adiposis in the liver.

At the end of our experiment, we were interested in determining the ultimate fate of the rats born of mothers exposed to a diet very rich in fats.

For these purposes we used five other female rats, who had been kept on a normal diet (break, milk, water) and three female rats on a normal diet, with the addition of 4-5 g of pork fat to their daily meal throughout the period of pregnancy and of lactation – in all from 2½ months to approximately 3½ months. In all cases lactation lasted from 25 to 27 days. First one of the control rats gave birth (5 September), followed by two of the rats on the high-fat diet (7 November and 19 December), then one of the controls (27 December), and finally the last experimental rat (19 March).

*I*st *LOT*

First control litter (delivery 5 September). – Six vivacious babies in excellent general condition, which eagerly hastened to suck and displayed a rapid growth, without any disturbance or alterations of a general or local nature. Their eyes opened after 13-14 days, the first soft hair appeared after 10-11 days, and their subsequent growth was not subject to any irregularities. On October 2, the suckling babies killed each other. Each weighed 35, 35, 34, 35, 34, and 34 g (average weight 34.5 g). No external or internal alterations were noted. The livers were pale pink in color, of normal external surface and size, of normal consistency. Each weighed 1.70, 1.65, 1.30, 1.35, 1.48, and 1.53 g (average weight 1.50 g). The histological examination did not show anything abnormal.

Second control litter (delivery 27 December). – Six well-nourished babies, with the appearance of soft hair after 10-11 days and opening of the eyes after 13-14 days. Each weighed 33, 35, 36, 34, 34, and 36 g (average weight 34.6 g). The livers were normal in appearance, color, and consistency, weighing respectively 1.62, 1.65, 1.66, 1.63, 1.63, and 1.66 g (average weight 1.65 g). Throughout the entire period of lactation, there were no alterations and the histological examination of the liver produced completely normal findings.

*II*nd *LOT*

First litter of rats bred by a mother on a diet of pork fat (delivery

7 November). Six babies whose condition did not differ from that described with respect to the newborns from the rats on a fat diet in the preceding lot. The mother ate regularly throughout the lactation period lasting 27 days, but the babies displayed a stunted growth. The first fine hair appeared after 12-13 days, The eyes opened after 19-20 days. One baby died at the age of 20 days. There were no skin alterations, On December 4, three suckling babies killed each other. Two females continued to live, but then died in a run-down state after approximately three months. They weighed respectively 20, 21, and 23 g; average weight was 21.3 g. The livers were yellowish-gray in color, soft upon palpation, oily and of dull appearance in the area of the incision. They weighed respectively 90 cg, 1 g, and 1.10 g (average weight 1 g). The histological examination showed a conspicuous adiposis of the hepatic cells quite a bit more accentuated than in the livers of the newborns.

Second litter of rats bred by a mother on a pork fat diet (delivery 19 December). – Six babies in a poor state of nutrition, cyanotic in color, with parched, wrinkled skin. The growth was stunted. After 10-11 days of life, all of these babies began to display skin alterations at the distal end of the tail: desquamation of the epidermis and drying of the dermis, which assumed a uniform blackish color. As the lesions proceeded towards the root of the tail, those parts of the dermis initially sickened continued to become detached in the form of scabs. Three newborns died 4-5 days after the appearance of this cutaneous alteration. The three surviving rats, whose first hair had appeared after 13-14 days, opened their eyes after 20-21 days. In these rats, the tail lesions were limited to the distal 2/3rd of the tail, restricted by a ring of newly produced connective tissue. They killed each other after 26 days. Each weighed 25 g and did not display any alterations outside of that described on the tail. There was little consistency in the livers, which were yellowish-pink in color and weighed 1.10 g on an average. The histological examination of the hepatic cells showed a scattering of granules and drops of fat, especially at

the periphery of the acini. However, the protoplasm of the hepatic elements was well-preserved, and the nuclei had not been extensively displaced.

Third litter of rats bred by a mother on a diet of pork fat (delivery 19 March). – Eight babies in fair health, whose growth was a little less stunted than the other babies from mothers on a pork fat diet. The first hair appeared after 12-13 days. The eyes opened after 18-20 days. There was no disturbance during the period of growth, nor any skin alteration. The lactation period lasted 25 days. At this age, the babies killed each other. Each one weighed respectively 20, 26, 22, 28, 26, 26.5, and 14 g (average weight 23.5 g). Their livers were yellowish in color, of diminished consistency, and with a turbid incision area, weighing respectively 95 cg, 1.20 g, 90 cg, 1.35 g, 1.20 g, 1.25 g, 1.10 g, and 50 cg (average weight 1.50 g). The histological examination showed the presence of a small quantity of granules and drops of fat scattered sparsely throughout the hepatic cells, which however did not appear to be structurally altered.

Based on the observation of these litters, we may confirm the differences regarding the weight and the general conditions existing at birth among newborns from rats on a normal diet and newborns from rats fed with pork fat. In addition, a diversity was observed in the development. We see that while the control sucklings grew normally, the others almost always displayed a stunted growth, with parched and wrinkled skin, a cyanotic color, and sluggish movements. The controls began to grow fine hair after 10-11 days and opened their eyes after 13-14 days, while the other rats began to grow hair after 13-14 days and opened their eyes after 18-21 days. Some died after a few days of life and the survivors, which killed each other after 25-27 days of nursing, proved to have a substantially lower weight than the babies from rats having been fed a regular diet. While the control babies varied in weight between 35 and 36 g (average weight 34.55 g), the other babies ranged in weight from 20-28 g (average weight 23.26 g). Differences were also observed upon macroscopic and

microscopic examination of the liver. The liver of the controls was normal in appearance, color, and consistency, with an average weight of 1.57 g, and did not display an abnormal fat deposit in the hepatic cells. On the other hand, the liver of the suckling babies from females on fat diets, in addition to having a uniformly grayish-yellow color, of limited consistency, with an oily and dull appearance in the area of the incision, and averaging 1.20 g in weight, displayed an accentuated state of adiposis upon histological examination. Granules and droplets of fat appeared to be scattered especially at the periphery of the acinus in the hepatic cells. The suckling babies from the second litter, as was mentioned, subsequently displayed a cutaneous alteration on the tail, which was identified as a characteristic necrosis upon histological examination.

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R. CLINICA PEDIATRICA DELL'UNIVERSITA' DI MODENA
Diretta dal Prof. G. DE TONI

Le ripercussioni sulla prole di un'alimentazione materna troppo ricca di grassi

Nota I.

Ricerche su ratte iperalimentate con grasso di maiale durante la gravidanza e l'allattamento

Dott. S. SANTYAN Y VELASCO Dott. G. PANCALDI
Assistenti Volontari

(*in redazione il* 18-4-40)

Il frequente riscontro nei bambini neonati e lattanti che affluiscono all'ambulatorio della nostra Clinica, di un notevole aumento di volume del fegato e il facile insorgere in essi, di disturbi legati a insufficienza funzionale epatica, sia spontaneamente che in occasione di svariate malattie, ci hanno indotto ad occuparci in modo particolare dell'argomento.

È verosimile che in ciò abbia parte l'eredità neuroartritica; che si tratti cioè di un epatismo congenito familiare; ma ci siamo posti il problema, se il largo uso di grassi nell'alimentazione, in questa provincia, non possa avere un'importanze degna di essere messa in luce.

Abbiamo inoltre potuto constatare in molti bambini la frequenza della diatesi essudativa con le svariate manifestazioni cutanee e generali ad essa legate, e sappiamo che secondo CZERNY, il componente capace di svelare lo stato diatesico latente, provocando manifestazioni di malattie, è costituito appunto dai grassi. È noto come la somministrazione soverchiamente abbondante di grassi animali possa portare a lesioni eczematose. Tra la diatesi essudativa e quella neuroartritica esistono del resto rapporti tanto stretti che spesso si ha sovrapposizione delle manifestazioni dell'una e dell'altra (fenomeni di epatismo, calcolosi epatica e renale, seborrea, intertrigine, eczemi, gotta, asma, adiposità ecc.). Anzi la diatesi essudativa del lattante e del piccolo bambino si continuerebbe nella diatesi neuroartritica del bambino più grande e dell'adulto (Francioni e Pacchioni).

Prima di esporre i risultati e lo scopo delle ricerche che formano oggetto di questa nota crediamo necessario ricordare in breve come avviene il metabolismo dei grassi secondo le più recenti vedute.

I grassi alimentari sono notoriamente costituiti da grassi neutri rappresentati da esteri di acidi grassi con glicerina. In questi la glicerina è combinata con tre radicali acidi e per ciò si parla di trigliceridi. I più comuni trigliceridi, quelli cioè che si trovano nel tessuto sottocutaneo e in altri depositi, sono miscele di tripalmitina, di tristearina e trioleina. Si trovano inoltre nei grassi, gliceridi misti, nei quali i gruppi alcoolici della glicerina sono saldati con due o tre diversi radicali di acidi grassi.

Come sappiamo, una quota di grassi è quasi indispensabile nella dieta, anzitutto perchè essi costituiscono un prezioso materiale di riserva e poi perchè sono gli alimenti capaci di svolgere il maggiore valore calorico col minimo volume (9,3 calorie per gr.). Alcuni grassi inoltre sono vettori di sostanze accessorie o vitamine ad azione molto importante nell'organismo. Secondo Starling il 30-35 % e più di calorie quotidiane nei bambini dovrebbe essere esplicato da sostanze grasse.

Il grasso viene assorbito dal tubo digerente, soprattutto come sapone alcalino e glicerina e nella parete intestinale stessa avviene la ricomposizione del grasso neutro il quale per mezzo dei vasi chiliferi arriva al sangue. Una parte di questo grasso neutro già nel sangue, viene scisso da lipasi circolanti e l'acido grasso che ne risulta va a far parte di elevati complessi molecolari, come sono le lecitine, desaturato e di nuovo ossidato. Un'altra parte invece, arrivata ai depositi, viene nuovamente scissa e indi risintetizzata da lipasi, molto abbondanti specialmente nei tessuti di maggiore importanza per il metabolismo dei grassi. Quando i grassi di deposito devono venire rimossi sono ancora le lipasi che li scindono o di ricostruiscono, finchè il grasso neutro arrivato nei luoghi di consumo, viene definitivamente trasformato in acido grasso e glicerina. La glicerina è bruciata e può dare zucchero, che sarà depositato sotto forma di glicogeno. Più complesso invece è il destino degli acidi grassi. Questi appartengono in gran parte alla serie satura ($C_nH_{2n}O_2$) qualcuno a quella non satura (C_nH_{2n}-2 O_2). La differenza tra acidi grassi saturi e non saturi sta nel fatto che in questi ultimi si trovano dei doppi legami nella catena aperta di atomi di carbonio, dovuti alla mancanza di atomi di idrogeno. Ora questi doppi legami possono aprirsi e fissare idrogeno oppure elementi capaci di sostituirlo, come gli alogeni.

Nel ricambio intermediario si formano soprattutto acidi grassi a numero pari di atomi di carbonio, di cui i più diffusi sono l'acido palmitico e l'acido stearico, che sono anche i costituenti principali dei grassi animali. Anche gli acidi non saturi però hanno molta importanza biologica. Di essi il principale è l'acido oleico. Esistono inoltre acidi grassi con molti doppi legami, quindi ancora meno saturi dell'acido oleico, come l'acido linoleico e linolico dell'olio di semi di lino. Gli acidi grassi saturi, secondo la nota teoria di *Knoop* e *Dakin* vengono attaccati con il fenomeno della beta-ossidazione unilaterale, iniziantesi probabilmente nel fegato (*Leathes, Meyer, Sinclair*) con caduta successiva di due in due atomi di carbonio. Il distacco dei due atomi di carbonio per volta avviene dunque per ossidazione in corrispondenza del beta-carbonio, ossia del secondo C della catena a partire dal carbossile. *F. P. Mazza, Verkade* ed altri AA. hanno studiato anche la omega-ossidazione e sono riusciti a dimostrare che, accanto alla via classica di degradazione per beta-ossidazione unilaterale, gli acidi grassi saturi vengono disintegrati « certamente anche per beta-ossidazione in omega, seguita poi da beta-ossidazione bilaterale degli acidi dioici formati ». E gli acidi grassi insaturi? Il

loro catabolismo non è ancora bene conosciuto. *Leathes e Raper* sostengono la scissione di questi per ossidazione, in causa del loro debole legame. Ciascun frammento verrebbe poi degradato per conto proprio. *Verkade* invece pensa che anche in questo processo abbiano gran parte la beta e la omega-ossidazione, di cui si è detto a proposito degli acidi saturi. Pochi anni or sono *Knoop, Oppenheimer e Wieland* ammisero che l'acido succinico avesse parte importante nel metabolismo intermediario degli acidi grassi, ma non si sapeva, se questo acido, diffuso anche nei muscoli, nel fegato ecc. provenisse dagli acidi grassi stessi, dagli idrati di carbonio o dagli aminoacidi. La teoria di *Verkade* sul catabolismo degli acidi grassi saturi per omega-ossidazione, seguita da beta-ossidazione bilaterale, permetterebbe di considerare gli acidi grassi saturi dei grassi alimentari, come « sostanze madri molto probabili, se non certe dell'acido succinico ».

Anche nella digestione dei grassi il fegato ha una notevole importanza, sia come ghiandola produttrice di bile, la quale per mezzo dei sali biliari favorisce la scissione degli acidi grassi e della colesterina, che per la funzione di deposito da esso esercitata anche per queste sostanze alimentari, oltrechè per le proteine e soprattutto per gli idrati di carbonio. Abbiamo inoltre già detto che la prima fase della ossidazione degli acidi grassi sarebbe l'insaturazione e che questo processo avverrebbe nel fegato. Il deposito ed il consumo dei grassi, come anche quello degli idrati di carbonio è influenzato da secrezioni ormoniche (tiroidee, ipofisarie, genitali) e dall'azione di vitamine. *Frank* nel 1923, *Frontali* nel 1924 e *Careddu* nel 1928 hanno potuto dimostrare che il complesso B, e più precisamente la vitamina B_2, idrosolubile, termolabile, favorisce l'utilizzazione e la tolleranza dei grassi alimentari negli organismi giovani ed ha azione elettiva sullo sviluppo.

Dopo questi brevi cenni generali veniamo ad occuparci più particolarmente dei caratteri dei principali grassi ed olii che più comunemente si usano nella alimentazione. Per maggiore chiarezza riuniamo i dati dei principali grassi ed olii in una tabella, mentre per la costituzione chimica del grasso di maiale, di cui ci siamo serviti per questa prima serie di ricerche, riportiamo dati più dettagliati, gentilmente segnalatici dal Regio Istituto di Chimica Biologica di Napoli, diretto dal Prof. Quagliarello, al quale rendiamo vivi ringraziamenti.

I valori medi sono tratti da analisi fatte sul grasso perirenale, sul pannicolo adiposo etc.

Il tessuto adiposo del maiale contiene: acqua 6,44 % - connettivo 1,35 %, - grasso 92,21 %. Nella composizione del tessuto adiposo dei maiale, vediamo quindi che a differenza di altri tessuti adiposi (di bue ad esempio) il grasso è in percentuale assai elevata. Il grasso tratto poi dal tessuto adiposo è così composto: carbonio 76,54 %, idrogeno 11,94 % ossigeno 11,52 %, punto di fusione 42-43°. Esso è costituito essenzialmente di trigliceridi, dell'acido oleico, stearico e palmitico; in piccola quantità è presente anche l'acido linoleico: I trigliceridi solidi sono rappresentati secondo *A. Bömer* da alfa-palmitodistearina (P. F. 68,5) e stearodipalmitina (P. F. 58,2). Secondo *Amberger* da beta-palmitodistearina.. La tristearina non sembra essere stata messa in evidenza. Riportiamo ancora le percentuali dei vari trigliceridi della sugna secondo *Bömer e Amberger*:

beta-palmito-alfa. alfa-distearina	circa 3 %
beta- stearo-alfa. alfa-dipalmitina	» 2 %
alfa-oleo-alfa. beta-distearina	» 2 %
alfa-palmito-alfa. beta-diolcina	» 82 %
beta-oleo-alfa palmito-alfa-stearina	» 11 %
Colesterina:	0,2-0,3 %

Contenuto percentuale di acidi grassi:

acido oleico	50-60 %
acido palmitico	21,6-32,8 %
acido linoleico	10 %
acido stearico	7,8 % fino a 15,4 %
Indice di saponificazione	195°
Numero di Jodio	76,5

Peso spec. a 15°	*Punto di fus. acidi grassi*	*Num. di Sapon.*	*Num. di Iodio*	*Num. degli acidi fissi*	*Num. degli acidi volatili*
Olio d'olive 0,913-0,919	22-28	185-196	79-88	95-96	0,3
Olio di arachidi 0,916-0,920	27-33	185-197	83-103	95-96	1
Olio di colza e di ravizzone 0,913-0,918	17-24	167-180	94-106	95-96	0,5
Olio di lino 0,930-0,937	17-24	187-195	164-194	95-96	—
Olio di cotone 0,922-0,931	34-40	190-198	101-117	95-96	—
Burro (a 100°) 0,865-0,868	38-45	220-232	26-40	85-91	25-30
Oleomargarina 0,924-0,930	40-43	192-200	42-55	95-96	0,4-1
Grasso di bue (sevo) 0,943-0,953	40-49	193-202	36-56	95-96	0,5
Grasso di maiale (sugna) 0,931-0,938	36-48	195-200	46-70	95-96	—
Grasso di Montone 0,937-0,961	44-52	192-197	32-46	95-96	—

Tabella secondo i dati del Trattato di Chimica Analitica di Villavecchia.

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Abbiamo dato inizio alle nostre ricerche il 30 maggio 1938 con l'allevamento di 5 giovani ratte (al momento dell'inizio dell'esperienza esse avevano un'età di circa due mesi), provenienti tutte da un'unica nidiata. A due di queste ratte veniva data un'alimentazione a volontà di pane, latte ed acqua; invece per l'altro lotto, di tre ratte, alla alimentazione pure a volontà di pane, latte ed acqua si aggiungevano quotidianamente 4-5 gr. di grasso di maiale globalmente. Abbiamo anche sottoposto alla alimentazione con eccesso di grassi un'altra ratta già adulta, tenuta nelle stesse condizioni delle altre. Quando iniziammo le nostre esperienze le prime due ratte, quelle cioè del lotto di controllo pesavano rispettivamente gr. 53 e gr 54; le altre pesavano gr. 51, gr. 55, gr. 58. Tutte quindi erano ancora in via di rapido accrescimento. La ratta adulta pesava al momento dell'inizio delle esperienze gr. 130. Un unico maschio del peso di gr. 105 veniva alternativamente tenuto una settimana con le due ratte di controllo ed una con le ratte alimentate con grasso di maiale.

La prima osservazione che ci fu dato di fare fu che alcuni giorni dopo l'inizio dell'alimentazione con sostanze grasse, le tre ratte che si alimentavano dapprima avidamente, incominciarono a perdere l'appetito, a dimagrire, a starsene accovacciate in un angolo della loro gabbia. Presentavano inoltre il muso un po' gonfio, le palpebre assai edematose, arrossate, con le ciglia più rade, il pelo arruffato e, fatto caratteristico, gli organi genitali esterni si notavano molto arrossati e rigonfi. Il maschio pure, dopo tre o quattro giorni di permanenza con queste ratte, presentava gli stessi disturbi, con cospicuo rigonfiamento dello scroto e dei testicoli e priapismo. Questo insieme di disturbi si attenuava fino a scomparire dopo 9-10 giorni, per ripresentarsi a periodi e nelle femmine in forma sempre più attenuata. L'estro delle ratte, come pure la loro attività genetica, ed anche l'attività sessuale del maschio, non furono per nulla alterati, come si può dedurre dal fatto che si ebbe il parto di una di queste, quasi contemporaneamente a quello di una femmina ad alimentazione normale.

Alcuni tra i disturbi da noi osservati furono già descritti nei ratti, da Frank e da Frontali, e attribuiti dal primo ad una iperavitaminosi A, dal secondo ad una parziale avitaminosi B, dato che furono ottenuti, sia in ratti alimentati con grassi ricchi di vitamina A, che in ratti nutriti con grassi privi di questo fattore (olo di soia). Recentemente però W. Weslaw-Wronski, A. Wroblewski e B. Wroblewski hanno descritto un complesso di sintomi del tutto identici a quelli in parte da noi osservati, accompagnantisi anche ad alterazioni scheletriche (fragilità, assottigliamento delle

ossa) dimostrate radiograficamente, in ratti tenuti in iperavitaminosi A, sia per via enterale, che per via sottocutanea e percutanea. Le nostre esperienze non avevano per scopo osservazioni dirette sugli animali adulti; ci limitiamo perciò a segnalare di sfuggita questo insieme di disturbi, senza per altro trattenerci a darne l'interpretazione.

Nostro scopo, come risulta dal titolo del lavoro, era di indagare le eventuali ripercussioni sulla prole di un'alimentazione troppo ricca di grassi. Noi veniamo subito perciò a prendere in esame le osservazioni da noi fatte sulle nidiate provenienti dalle ratte messe in esperienza. Dal punto di vista cronologico diremo che dopo circa due mesi dall'inizio delle esperienze (30 maggio) si ebbe il parto quasi contemporaneo di una ratta a dieta normale (26 luglio) e di una ratta ad alimentazione con aggiunta di grassi (27 luglio). Successivamente partorirono la seconda ratta di controllo (25 agosto), la seconda (3 settembre) e la terza (8 settembre) ratta a dieta di grassi e infine si ebbe il parto della ratta adulta.

I. LOTTO

Prima nidiata di controllo (parto 26 luglio). — 6 neonati di buone dimensioni, assai vivaci nei loro movimenti, di colorito roseo. Si uccisero madre e neonati. Pesi singoli dei neonati: gr. 5,87 - 5,88 - 5,12 - 5 - 5,94 - 5; peso medio gr. 5,47. La ratta dopo il parto pesava gr. 130 (da gr. 54). Il fegato della ratta pesava gr. 5,87, era di aspetto colorito e consistenza normali. I fegati dei neonati pure di aspetto normale pesavano singolarmente: ctgr. 28 - 28 - 27 - 28.8 - 30 - 22; peso medio ctgr. 27.3. L'esame istologico sia del fegato della ratta che di quello dei neonati fissati in formalina e poi colorati con Sudan III, non mise in evidenza un'abnorme presenza di grasso. Si notava soltanto nelle cellule epatiche qualche scarsa gocciolina di grasso, che rappresenta un reperto del tutto normale.

Seconda nidiata di controllo (parto 25 agosto). — 6 neonati in buone condizioni generali e di nutrizione. Su uccisero madre e neonati. La prima pesava gr. 135 (da gr. 53) e ciascun neonato gr. 6 - 5,90 - 6 - 5,87 - 5,95 - 6; peso medio gr. 5,96. Il fegato della ratta di colorito aspetto e consistenza normali pesava gr. 5,90. Quelli dei neonati pure normali erano del peso rispettivo di ctgr. 32 - 28 - 29 - 28 - 30 - 30; peso medio ctgr. 29,5. L'esame istologico non dimostrò alcun fatto anormale.

II. LOTTO

Prima nidiata di neonati da madre ad alimentazione di grassi (parto 27 luglio). — 5 piccoli che si presentarono poco vivaci di colorito assai cianotico. Si uccisero madre e neonati; la prima pesava gr. 90 (da gr. 51) e i piccoli rispettivamente gr. 4,80 - 5,60 - 4,70 - 4,80 - 4,60; peso medio gr. 4,90. Il fegato della ratta del peso di gr. 5,87 si presentava abbastanza voluminoso, di colorito piuttosto giallastro, qua e là tendente al cenerognolo, di consistenza alquanto diminuita, caratteri tutti che apparivano anche alla superficie di taglio. I fegati dei neonati di colorito giallognolo o cenerognolo, erano di

consistenza molliccia, facilmente spappolabili, del peso rispettivo di ctgr. 20 - 20 - 19,8 - 19,5 - 19,8; peso medio ctgr. 19,8. Nelle logge perirenali della ratta, come pure lungo il mesentere e in grado minore anche nel sottocutaneo, si notava la presenza di abbondante deposito di grasso avente un aspetto mucillaginoso e un colorito biancastro, simile al grasso che la ratta aveva ingerito. All'esame istologico, sia nel fegato della madre, che in quello dei neonati, si mise in evidenza un più o meno spiccato stato di adiposi. Le cellule epatiche apparivano cosparse, specie alla periferia del protoplasma, di numerosi granuli e gocciole di grasso di varia grandezza, qua e là riunite così da formare vere e proprie vescicole adipose. Il nucleo di parecchie cellule, appariva spostato alla periferia della cellula stessa e spesso il protoplasma era ridotto ad un sottile orlo.

Seconda nidiata di neonati da madre ad alimentazione di grassi (parto 3 settembre). — Otto piccoli di dimensioni molto inferiori a quelle degli altri, di aspetto sofferente, poco vivaci, intensamente cianotici, con la cute arida, raggrinzita. Si uccisero madre e neonati. La prima pesava gr. 100 (da gr. 58). Ciascun piccolo era del peso rispettivo di gr. 3,60 - 3,60 - 3,40 - 3,50 - 3,20 - 3,40 - 3,50 - 3,40; peso medio gr. 3,40. Il fegato della ratta pesava gr. 6,40 e si presentava voluminoso, giallastro a tinta uniforme, piuttosto molle alla palpazione e la superficie di taglio appariva assai umida, non granulosa, di colorito rosso-giallognolo sporco. I fegati dei neonati erano piccoli, di colorito roseo giallastro, non roseo come nei controlli, di consistenza molle, spappolabile, del peso rispettivo di ctgr. 15 - 19 - 12 - 19 - 15 - 12 - 19; peso medio ctgr. 16 circa. Alla superficie di taglio non si rilevavano acini, ma questa appariva compatta, torbida. All'esame istologico si notò abnorme presenza di grasso sia nel fegato della madre che in quello dei neonati. Anche nelle regioni pararenali, lungo il mesentere e nel sottocutaneo di questa ratta, si notò la presenza di notevoli quantità di grasso, mucillaginoso, biancastro.

Terza nidiata di neonati da madre ad alimentazione di grassi (parto 8 settembre). — Sei piccoli in condizioni di nutrizione pure scadenti, con la cute cianotica, raggrinzita e secca. Si uccisero madre e neonati, questa pesava gr. 98 (da gr. 55). Ciascun piccolo era del peso di gr. 3,60 - 3,80 - 3,70 - 3,60 - 3,40 - 3,50; peso medio 3,6 gr. Il fegato della ratta pesava gr. 6,20 e presentava i caratteri già descritti a proposito della precedente. Quello dei neonati era piccolo, giallastro, poco consistente di peso rispettivo di ctgr. 17 - 16 - 18 - 19.3 - 17.9 - 15 (peso medio ctgr. 17,3). Sia nel fegato della ratta che in quello dei neonati si mise in evidenza all'esame istologico un accentuato stato di adiposi.

Quarta nidiata di neonati da madre ad alimentazione di grassi (parto 10 settembre). — La ratta che pesava all'inizio gr. 130, durante la gravidanza presentò gli stessi disturbi e le stesse alterazioni delle precedenti. 10 piccoli in condizioni di nutrizione scadenti, di colorito molto cianotico. Si uccisero madre e neonati. Questa pesava gr. 150. Il suo fegato era del peso di gr. 8, si presentava voluminoso, di colorito rosso-giallastro, a tinta uniforme, poco consistente alla palpazione, molle al taglio, di cui le superfici apparivano assai umide, torbide, opache. Non si rilevavano acini. I neonati pesavano gr. 4,30 - 4,20 - 4 - 5 - 4,20 - 5 - 4 - 4,70 - 4 - 4,20. Peso medio gr. 4,25. I loro fegati avevano un colorio rosso-nerastro, erano molliccì, spoppolabili e pesavano rispettivamente ctgr. 23 - 23 - 23 - 22 - 24 - 22 - 23 - 22 - 24 - 22; peso medio ctgr. 23. L'esame istologico sia del fegato della ratta che di quello dei neonati dimostrò un accentuato stato di adiposi.

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Come si è già detto, abbiamo incominciato le nostre esperienze con l'allevamento di due lotti di ratte gravide. Ciascuna di queste, eccettuata l'ultima, era ancora nel periodo di rapido accrescimento poichè dal peso di gr. 51-58, salì dopo il parto a gr. 90-135, quindi a quasi il doppio o più del peso iniziale. Abbiamo già accennato alle alterazioni presentate dalle ratte tenute a dieta ipergrassa. Anche il loro accrescimento fu assai stentato. Mentre quelle a dieta normale pesavano dopo il parto gr. 130 e gr. 135, queste raggiunsero soltanto il peso rispettivo di gr. 90-100-98. Evidentemente la dieta ricca di grassi, provocando i fenomeni sopra descritti aveva dannosamente influito sull'accrescimento degli animali.

I fegati dei ratti di controllo, normali per aspetto, dimensioni, colorito e consistenza, come pure nella loro struttura istologica, pesavano gr. 5,87 e gr. 5,90 (peso degli animali gr. 130-135), mentre quelli delle ratte a dieta ricca di grassi si presentavano voluminosi, di colorito uniformemente giallastro, di consistenza diminuita, con la superficie di taglio

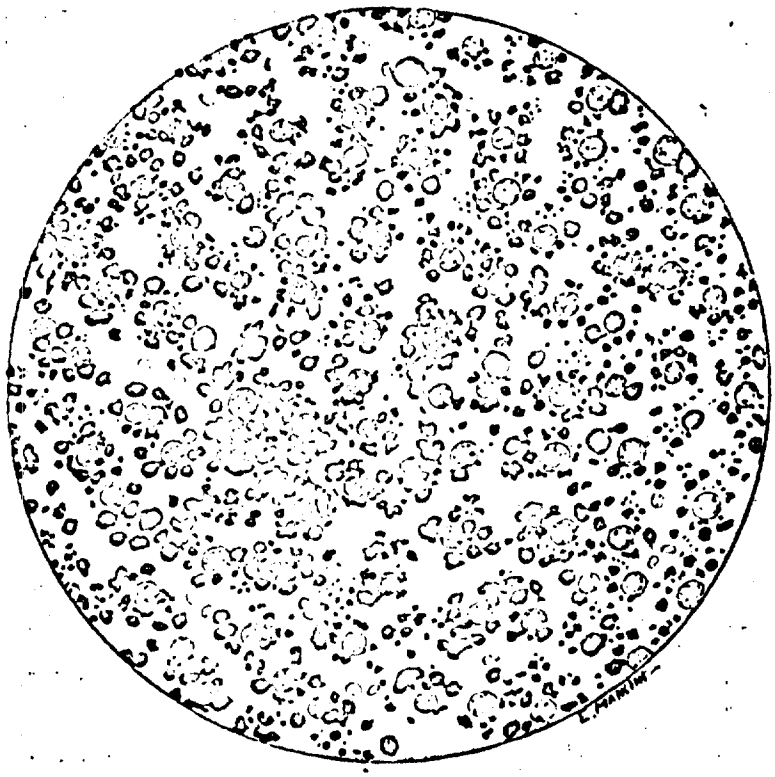

Fig. 1.

assai umida, lucida, untuosa. Su questa inoltre non si notava la forma degli acini. Il loro peso era di gr. 5,87-6,40-6,20-8) peso delle ratte grammi 90-100-98-150) tanto più sproporzionato quindi a quello degli animali, quanto più a lungo questi erano stati alimentati con grassi. L'esame istologico eseguito allo scopo di mettere in evidenza un'eventuale abnorme presenza di grasso nelle cellule epatiche (pezzi fissati in formalina e colorati poi con Sudan III) dimostrò sempre un più o meno accentuato stato di adiposi (figura 1).

L'adiposi del fegato di origine infiltrativa come è più probabile (*Lebedeft*, *Rosenfeld*, *Cavazza* etc.) o degenerativa (*Mavrakis*, *Wirchow*, etc.) è in genere come afferma *Rondoni* « proporzionale alla ricchezza di deposito di grasso dell'animale . Abbiamo visto che nel sottocutaneo, ma soprattutto nelle logge renali, e lungo il mesentere delle ratte ad alimentazione speciale, si notava la presenza di notevole quantità di un grasso avente aspetto e colorito caratteristici. Si tratta infatti di deposito di grasso alimentare, e che questo possa essere immagazzinato direttamente dall'organismo, risulta dalle note esperienze di *Pettenkofer* e *Voit* ed ancor meglio da quelle di *J. Munk* e di *Hoffmann*. Infatti mentre da molti si ammetteva soltanto l'origine endogena del grasso di deposito, poichè si pensava che quello alimentare venisse completamente bruciato, gli AA. sopra citati dimostrarono invece, mediante la somministrazione di molto grasso e poche sostanze proteiche a cani precedentemente fatti digiunare, così da impoverire la loro riserva di grasso, che, anche i grassi introdotti cogli alimenti, possono venire immagazzinati nei depositi adiposi. Secondo *Zummo* poi la temperatura esterna avrebbe molta influenza sulla costituzione del tipo di grasso di deposito.

Nessuna differenza si è rilevata riguardo al numero dei neonati ed all'epoca di nascita nei due lotti di ratte. Per quanto concerne invece le condizioni generali ed epatiche e lo stato di nutrizione dei neonati stessi, bisogna dire qualche parola di più. Le due ratte di controllo partorirono ciascuna sei piccoli, svelti nei loro movimenti, in condizioni generali ottime, bene nutriti, non presentanti alcuna alterazione cutanea, di colorito roseo, che si attaccarono subito al seno materno con avidità, come dimostrò la ripienezza di latte del loro stomaco il giorno dopo la nascita, quando vennero uccisi. Il loro peso oscillò da un minimo di gr. 5 ad un massimo di gr. 6, con una media di gr. 5,71. I fegati di questi neonati erano di colorito roseo, abbastanza consistenti, con un peso oscillante fra un minimo di centgr. 22 ed un massimo di ctgr. 30 (peso medio ctgr. 28,4). Il loro esame istologico dimostrò cellule epatiche integre, oppure contenenti nel loro protoplasma, scarsi granuli di grasso, i quali rappresentano un reparto del tutto normale (figura n. 2).

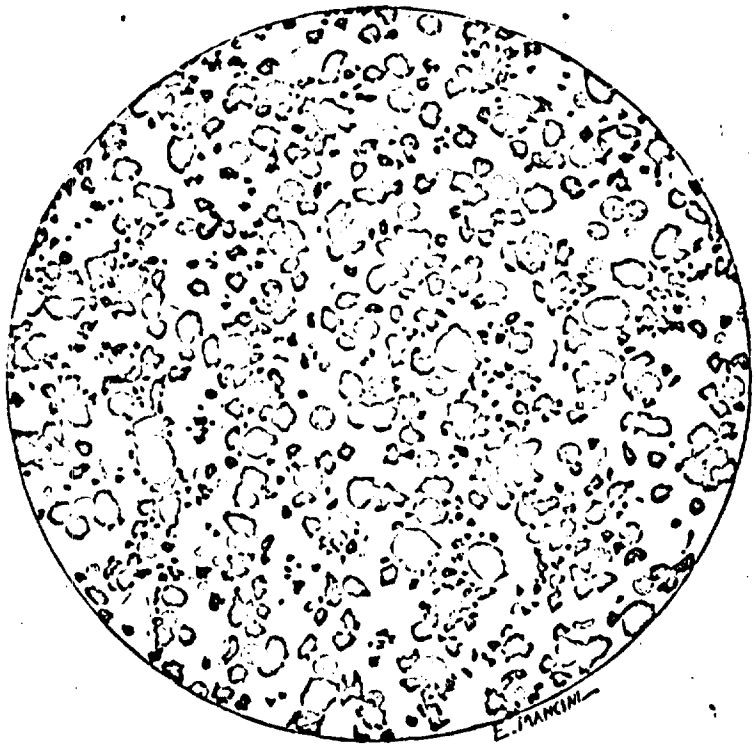

Fig. 2.

I neonati delle madri ad alimentazione ricca di grasso di maiale, specialmente quelli delle ratte tenute per lungo tempo (più di tre mesi) a dieta speciale, apparivano debilitati, poco vivaci nei movimenti, denutriti, piuttosto sofferenti, succhiavano il latte stentatamente, erano di colorito fortemente cianotico, avevano la cute arida e raggrinzita. Il loro peso oscillò fra gr. 3,20 e gr. 5,60 (soltanto uno però raggiunse il peso di gr. 5,60) con un peso medio di gr. 4,04. I neonati della prima e della quarta nidiata erano in condizioni meno scadenti di quelli delle altre due. Infatti le madri di essi si alimentarono a grassi di maiale soltanto circa due mesi, mentre per la seconda e terza ratta, tra l'inizio delle esperienze ed il parto intercorsero più di tre mesi.

Il fegato di questi neonati, appariva in genere piccolo, di colorito giallastro, di consistenza molliccia, non compatto, ma facilmente spappolabile, di peso oscillante tra ctgr. 12 e ctgr. 24, con un peso medio di ctgr. 19, quindi notevolmente inferiore a quello del fegato dei controlli.

L'esame istologico dei pezzi fissati in formalina e colorati poi con Sudan III mise in evidenza un più o meno spiccato stato di adiposi (fig. n. 3).

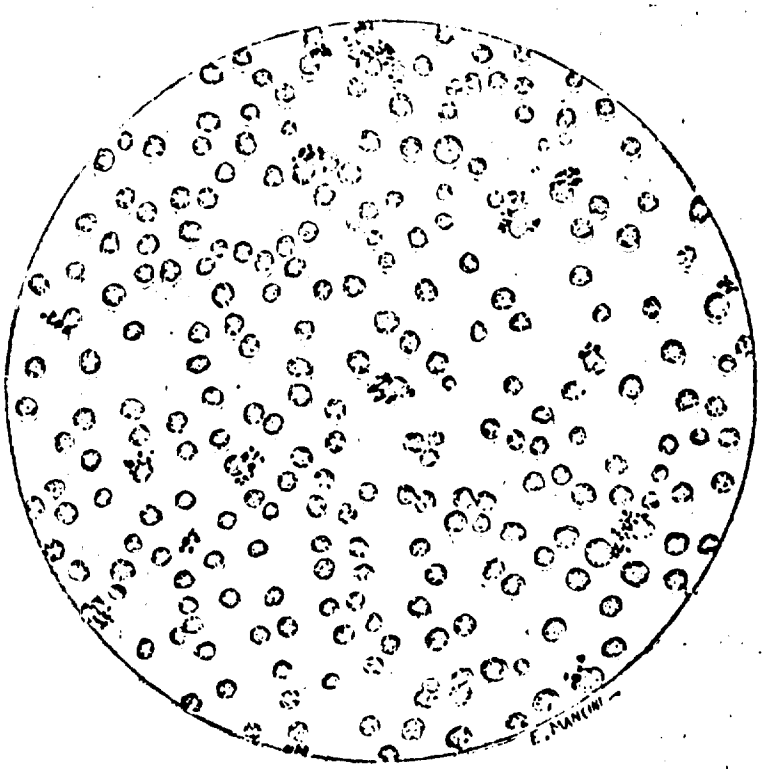

Fig. 3.

Le cellule epatiche apparivano disseminate di granuli e gocciole di grasso, specialmente abbondanti alla periferia del citoplasma, in qualche punto raggruppati così da formare vere e proprie vescicole adipose. Il nucleo delle cellule stesse appariva spesso spostato alla periferia e il protoplasma ridotto.

Prescindendo dunque dalle osservazioni incidentali e guardando soltanto ciò che a noi più interessa e cioè quale differenza esista per quanto riguarda condizioni generali ed epatiche, oltrechè eventuali svariate alterazioni o manifestazioni diatesiche tra i neonati da ratte ad alimentazione normale e quelli di ratte ad una nutrizione ipergrassa (grassi di maiale), dobbiamo dire che questa è stata per certi aspetti netta e chiara. Le ratte tenute ad una dieta ricca di grassi di maiale, oltre a presentare particolari e svariati disturbi ed avere un accrescimento piuttosto stentato,

hanno partorito piccoli in scadenti condizioni generali e di nutrizione, debilitati, con la cute arida e presentanti il fegato in più o meno spiccato stato di adiposi.

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Ai fini del nostro lavoro interessava però conoscere la sorte ulteriore dei ratti nati da madri sottoposte a dieta molto ricca di grassi. Siamo partiti per ciò da altre cinque ratte, di cui due tenute a dieta normale (pane, latte, acqua) e tre a regime comune, con aggiunta al pasto quotidiano di gr. 4-5 di grassi di maiale) durante tutto il periodo di gravidanza e quello di allattamento, in tutto da due mesi e mezzo a tre mesi e mezzo circa. L'allattamento durò in tutti i casi da 25 a 27 giorni. Dapprima si è avuto il parto di una ratta di controllo (5 settembre), indi di due femmine a dieta ipergrassa (7 novembre e 19 dicembre) poi di una di controllo (27 dicembre) e infine dell'ultima in esperimento (19 marzo).

I. LOTTO. — *Prima nidiata di controllo* (parto 5 settembre). — 6 piccoli in ottime condizioni generali, vivaci, che presero subito a succhiare con avidità e presentarono una crescita rapida, senza alcun disturbo nè alterazioni di ordine generale nè locale. Aprirono gli occhi dopo 13-14 giorni, emisero la prima peluria a 10-11 giorni di vita e anche in seguito l'accrescimento non subì alcuna irregolarità. Il 2 ottobre si uccisero i lattanti. Ciascuno di essi pesava gr. 35-35-34-35-34-34 (peso medio gr. 34,5). Non si notò alcuna alterazione esterna nè interna. I fegati erano di colorito roseo pallido, avevano una superficie esterna e di taglio normali, una consistenza pure normale. Pesavano ciascuno gr. 1,70-1,65-1,30-1,35-1,48-1,53 (peso medio gr. 1,50). L'esame istologico non dimostrò nulla di anormale.

Seconda nidiata di controllo (parto 27 dicembre). — 6 piccoli in buone condizioni di nutrizione, che emisero la prima peluria e aprirono gli occhi rispettivamente a 10-11 giorni e a 13-14 giorni. Ciascuno pesava gr. 33-35-36-34-34-36 (peso medio gr. 34,6). I fegati normali per aspetto, colorito, e consistenza, pesavano rispettivamente gr. 1,62-1,65-1,66-1,63-1,63-1,66 (peso medio gr. 1,65). Durante tutto il periodo di allattamento non si ebbe alcuna alterazione e l'esame istologico del fegato diede un reperto del tutto normale.

II. LOTTO. — *Prima nidiata di ratti allevati da una madre tenuta a dieta di grassi di maiale* (parto 7 novembre). — Sei piccoli in condizioni non diverse da quelle descritte a proposito dei neonati delle ratte a regime di grassi del precedente lotto. La madre si alimentò regolarmente durante tutto il periodo di allattamento durato 27 giorni, ma i piccoli ebbero un accrescimento assai stentato. Emisero la prima peluria a 12-13 giorni. Aprirono gli occhi a 19-20 giorni. Uno morì all'età di 20 giorni. Non presentarono alcuna alterazione cutanea. Il 4 dicembre tre lattanti vennero uccisi. Due femmine furono lasciate in vita, ma morirono poi in stato di deperimento dopo tre mesi circa. Pesavano rispettivamete gr. 20-21-23 (peso medio 21,3). I fegati erano di colorito giallastro cenerognolo, mollicci alla palpazione, untuosi e di aspetto opaco alla

superficie di taglio. Pesavano rispettivamente ctgr. 90 - gr. 1 - gr. 1,10 (peso medio gr. 1). L'esame istologico dimostrò una notevole adiposi delle cellule epatiche assai più accentuata che nei fegati dei neonati.

Seconda nidiata di ratti allevati da madre a regime di grassi di maiale (parto 19 dicembre). — Sei piccoli in condizioni di nutrizione scadenti, di colorito cianotico, con la cute arida, raggrinzita. L'accrescimento fu stentato. A 10-11 giorni di vita tutti questi piccoli incominciarono a presentare alterazioni cutanee alla estremità distale della coda: desquamazione dell'epidermide, essiccamento del derma, il quale assunse un colorito nerastro uniforme. A mano a mano che la lesione procedeva verso la radice della coda, le parti del derma ammalate per prime andavano staccandosi in forma di croste. Tre neonati morirono dopo 4-5 giorni dall'inizio di questa alterazione cutanea. I tre sopravvissuti, che avevano emesso la prima peluria a 13-14 giorni, aprirono gli occhi a 20-21 giorni. In essi le lesioni alla coda erano rimaste circoscritte ai due terzi distali di essa, limitate da un cercine di neoproduzione connettivale. Furono uccisi a 26 giorni di vita. Ciascuno pesava gr. 25, non presentava alcuna alterazione all'infuori di quella descritta alla coda. I fegati poco consistenti, di un colorito rosco-giallastro, avevano un peso medio di gr. 1,10. All'esame istologico le cellule epatiche apparivano disseminate di granuli e goccioline di grasso specialmente disposti alla periferia degli acini. Il protoplasma degli elementi epatici però, era ben conservato, ed anche i nuclei non erano considerevolmente spostati.

Terza nidiata di ratti allevati da madre ad alimentazione di grassi di maiale (parto 19 marzo). — Otto piccoli in discrete condizioni generali, che ebbero un accrescimento un po' meno stentato degli altri discendenti da madri ad alimentazione di grassi di maiale. Emisero la prima peluria a 12-13 giorni. Aprirono gli occhi a 18-20 giorni. Non ebbero alcun disturbo durante il periodo di accrescimento, nè alcuna alterazione cutanea. Il periodo di allattamento durò venticinque giorni. A tale età vennero uccisi. Ciascuno pesava rispettivamente gr. 20-26-22-28-26-26,5-25-14 (peso medio gr. 23,5). I loro fegati aventi un colorito giallastro, una consistenza diminuita, una superficie di taglio torbida, pesavano rispettivamente ctgr. 95 - gr. 1,20 - ctgr. 90 - gr. 1,35-1,20-1,25-1,10 - ctgr. 50 (peso medio gr. 1,50). All'esame istologico si dimostrò la presenza di una discreta quantità di granuli e goccioline di grasso sparsi nelle cellule epatiche, le quali non apparivano peraltro alterate nella loro struttura.

* * *

Dall'osservazione di queste nidiate si ebbe anzitutto la conferma delle differenze riguardo al peso ed alle condizioni generali esistenti alla nascit afra neonati da ratte ad alimentazione normale e quelli da ratte alimentat econ grasso di maiale; inoltre si rilevarono diversità nello sviluppo. Si vide che mentre i lattanti di controllo crescevano normalmente, gli altri avevano quasi sempre un accrescimento assai stentato, la loro cute era arida e raggrinzita, di colorito cianotico, i loro movimenti erano lenti. I primi si coprirono di peluria a 10-11 giorni e aprirono gli occhi a 13-14 giorni, mentre i secondi rispettivamente a 13-14 giorni e a 18-21 giorni. Taluni morirono dopo pochi giorni di vita ed i sopravvissuti, uccisi dopo

25-27 giorni di allattamento, si dimostrarono di peso assai inferiore a quelli provenienti da ratte ad alimentazione regolare. Mentre quelli di controllo ebbero il peso oscillante fra gr. 34-36 (peso medio gr. 34,55), quelli allo studio furono di peso nei limiti di gr. 20-28 (peso medio gr. 23,26). Anche all'esame macroscopico e microscopico del fegato si notarono differenze. Quello dei controlli normale per aspetto, colorito e consistenza, di un peso medio di gr. 1,57, non presentò un'abnorme deposizione di grasso nelle cellule epatiche; invece quello dei lattanti discendenti da femmine a grassi, oltre ad essere di colorito uniformemente giallo cenerognolo, poco consistente, con le superfici di taglio untuose, opache, di un peso medio di gr. 1,20, dimostrò all'esame istologico un accentuato stato di adiposi. Le cellule epatiche apparivano disseminate specie alla periferia dell'acino, di granuli e goccioline di grasso. I lattanti della seconda nidiata dimostrarono poi, come si è detto, un'alterazione cutanea alla coda, che all'esame istologico apparve una caratteristica necrosi.

RIASSUNTO

Questa nostra prima serie di ricerche, iniziate per studiare le ripercussioni sulla prole di un'alimentazione materna troppo ricca di grassi, ci ha permesso di rilevare, oltre a particolari disturbi ed alterazioni negli animali adulti a dieta ipergrassa, cospicue differenze riguardo alle condizioni generali ed epatiche e allo sviluppo fisico dei neonati e dei lattanti provenienti dalle ratte in esperimento rispetto a quelli nati dalle femmine di controllo.

RÉSUMÉ

Cette première série de recherches que nous avons entreprise pour étudier les répercussions sur l'enfant d'une alimentation maternelle trop riche en graisses nous a permis de constater non seulement des troubles particuliers et des altérations chez les animaux adultes à diète hyper-grasse, mais encore des différences remarquables en ce qui concerne les conditions générales et hépatiques et le développement physique des nouveau-nés et des nourrissons provenant des femelles des rats soumises à l'expérience par rapport aux petits nés des femelles de contrôle.

SUMMARY

This first series of researches, initiated to study the reflection on children of a maternal alimentation too rich in fats, allowed us to relief,

besides the special disturbances and alterations of an hyperfatty diet on adult animals, remarkable differences regarding the general and hepatic conditions and the physical development of the newborns and sucklings deriving from female rats under experiment in respect of those born from females acting as controls.

ZUSAMMENFASSUNG

Mit dieser ersten Versuchsreihe, welche den Zweck hatte die Folgewirkungen einer allzu fettreichen Nahrung der Mutter auf die Kinder festzustellen, wurden in bei fettreicher Diät gehaltenen erwachsenen Tieren nichtnur besondere Störungen und Veränderungen in diesen Tieren selbst nachgewiesen, sondern auch bedeutende Unterschiede, im Vergleich mit den Jungen der Kontrollweibchen, im Allgemeinzustand, in der Leberfunktion und in der Körperentwicklung der neugeborenen und der saugenden Ratten.

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Vinson-'44
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Growth, Reproduction, and Lactation in Rats Maintained Through Four Generations on Highly Purified Diets[1]

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Introduction

In the past few years conflicting results have been reported concerning the nutritional requirements of rats for reproduction and lactation. Although it is generally agreed that a diet adequate for growth does not necessarily have to be adequate for reproduction and lactation, opinions vary among investigators as to what constitutes a good highly purified ration that will enable an animal to pass through one or more normal cycles of growth, reproduction, and lactation. Several groups of workers have claimed moderate or good success with diets containing the established B complex vitamins in crystalline form. With such a diet Jukes (1) obtained some success on reproduction in rats although the growth rate was below normal. Unna (2), Unna and his co-workers (3), Henderson, *et al.* (4), and Folley, *et al.* (5) have been able to maintain rats on synthetic diets through at least three generations. However, lactation, for the most part, was poor, and average weaning weights of the surviving litters were low. Richardson, *et al.* (6) used a diet which they found to be excellent for growth and adequate for reproduction and lactation. In a subsequent communication (7), however, they stated that a supplement of liver extract was required for successful lactation. The importance of *p*-aminobenzoic acid and to a lesser extent of inositol was emphasized by Sure (8), who found that normal lactation was only obtained when these supplements were added to the diet. Similar conclusions were reached by Climenko and McChesney (9), except that their results indicated that inositol was the more important lactation factor.

[1] A preliminary report of this investigation has appeared (18).

In this laboratory two strains of rats have been kept on highly purified diets through four generations. The growth of these animals was excellent in every generation. Of particular interest was the observation that the mothers invariably lost considerable weight during the nursing period.

Experimental

The rats used in these experiments were of two kinds, belonging to the black or piebald Evans-Long strain, and to the Wistar strain.

Several diets were employed (Table I). Of those listed, only one (diet R-3) proved to be totally inadequate in supporting lactation. Animals on this ration suffered frequently from diarrhea. Of the other diets, those that contained a

TABLE I

Composition of Diets

Component	Diet					
	R-1	*R-2*	*R-3*	*R-4*	*R-5*	*R-6*
Purified Casein (Labco or Smaco)	30	30	30	30	30	30
Salts (Osborne and Mendel)	5	5	5	5	5	5
Ruffex[1a]	2	2	2	2	2	2
Lard	10	—	—	2	5	—
Crisco	—	8	—	8	10	10
Corn oil	—	2	10	—	—	—
Sucrose	53	53	53	53	48	53

Supplements were the same for all diets. They were per kilogram: thiamine, 20 mg.; riboflavin, 20 mg.; pyridoxin, 20 mg.; calcium pantothenate, 40 mg.; choline chloride, 500 mg.; α-tocopherol, 20 mg.; vitamin A and D concentrate, 40 mg.

[1a] α-Cellulose preparation obtained from Eimer and Amend, New York.

combination of lard and Crisco (diets R-4 and R-5) gave slightly better results in so far as average weaning weights of litters were concerned. Diet R-5 containing 15% fat was most frequently used in these experiments. Evans, *et al.* (10) and Maynard and Rasmussen (11) have shown that lactation improved when the fat content of the diet was increased.

In our early experiments the protein level of the diet was 20%. This ration was the same as diet R-1 except for the lowered protein content and resembled closely the diet employed by Richardson, *et al.* (6). Wistar rats, placed on this diet when 21 days old, grew at a normal rate, but complete failure met attempts to raise litters upon mating 20 females. Upon increasing the protein content to 30%, successful lactation was

obtained. These results are in agreement with those of Daggs and Tamboulian (12), and of Sure (13).

Four generations were raised on the highly purified diets. Table II presents the data on reproduction and lactation for both strains of rats used. It can be seen that the number of young weaned is significantly smaller for both strains of rats on the purified rations than the number weaned on the control diet of Purina dog chow. However, the growth of these animals was excellent throughout the four generations.

TABLE II

Data on Reproduction and Lactation

Strain	Generation	No. of Females	Litters born	Litters weaned	Young weaned	Litter size	Average weaning weight g.
Wistar	Parent	27	25	15	101	6.7	38.3
"	F_1	35	26	15	79	5.3	33.8
"	F_2	13	12	4	16	4.0	34.2
"	F_3	7	4	1	4	4.0	29.6
"	Total	82	67	35	200	—	—
"	Controls on Purina dog chow	15	14	11	79	7.0	34.5
Evans and Long	Parent	4	4	4	33	8.3	26.4
"	F_1	15	14	11	68	6.0	26.5
"	F_2	15	12	4	24	6.0	25.8
"	Total	34	30	19	125	—	—
"	Controls on Purina dog chow	10	10	8	75	9.4	25.3

Figs. 1 and 2 show typical growth curves of litters raised on the purified diets and on Purina dog chow. The Evans-Long strain rats grew at a rate equal to that of litter mates on the control diet. Also, the slopes of the growth curves for the parent and second filial generations are about the same. Similar results were observed with the Wistar rats.

A very important observation has been made during lactation. Invariably the mother lost considerable weight, particularly during the latter part of lactation. Immediately after separating the rat from the weaned litter, she regained sharply the weight lost. Fig. 3 shows the fluctuations in weight of a Wistar rat (F_1 generation) through a

period covering three successful pregnancies. It will be observed that soon after mating the sharp rise in the curve is due to pregnancy, while the precipitous drop corresponds to parturition. Then, in every case

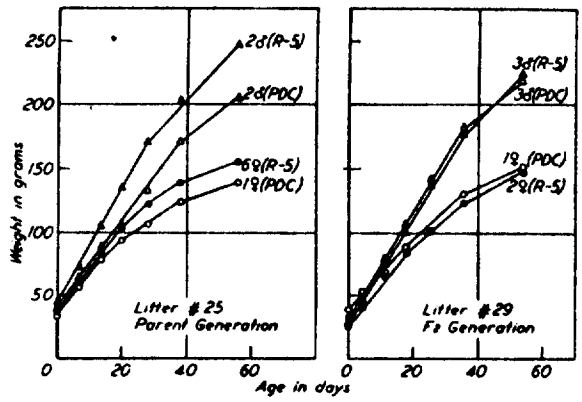

FIG. 1

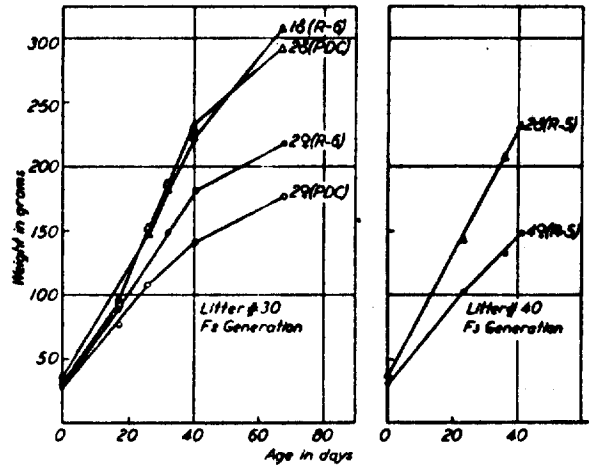

FIG. 2

there is a slight gain in weight during the first few days of lactation with a subsequent sharp drop for the remainder of the lactation period. Upon separating the mother from the young, the curve rises sharply denoting a gain in weight again. This cycle is repeated in the same manner for the other two pregnancies.

Under the conditions of our experiments the average loss in weight of Wistar rats during lactation was 40 g. In some cases the loss was much larger, reaching 70 g. This enormous loss occurred when a mother was taking care of a large litter consisting of eight or more young. For a 250 g. rat, such a drop in weight amounts to almost 30% of the body weight. A similar phenomenon was observed in the case of the Evans-Long rats except that the loss in weight during lactation was not quite as great. This difference may be due to the fact that an adult female of the Evans-Long strain weighs less than an adult Wistar female.

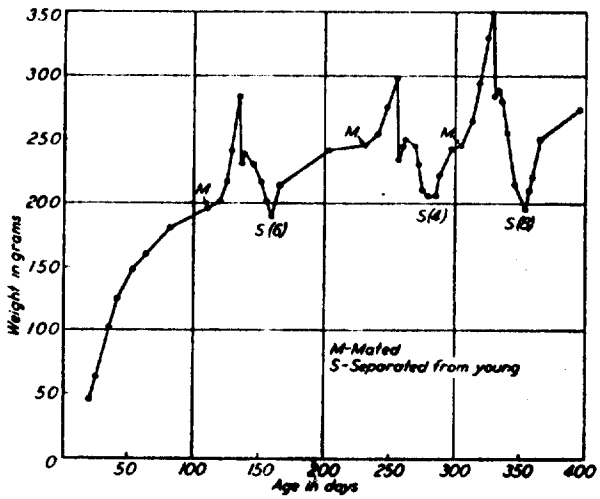

FIG. 3

Some other observations made on lactating rats kept on highly purified diets are worth recording. It was noticed that several Evans-Long rats ate their feces. Also, there was a frequent collapse of the mother in the late stages of lactation (15th–18th day). In such cases, either the litter was destroyed, or the mother died from complete exhaustion. These rats appeared very shabby and emaciated. Due to the constant nursing of their young, several mothers developed ulcerated mammary glands.

It was concluded from these observations that the diet was inadequate in supplying some constituent needed for maximum milk production. To rule out the possibility that one of the primary foods might have

been ingested in insufficient quantities, the following supplements were tested by supplying them *ad libitum* to lactating mothers: casein, sucrose, and lard. None of these improved the lactation performance of the rats. In fact, the mothers fed the lard supplement destroyed their young during lactation. These rats ate the lard ravenously, eating as much as 8 g. per day. Apparently the consumption of such large amounts of lard interfered with the ingestion of other food constituents.

Other supplements that were tried without any beneficial effect in preventing the high weight loss of lactating mothers were: Biotin, 0.1

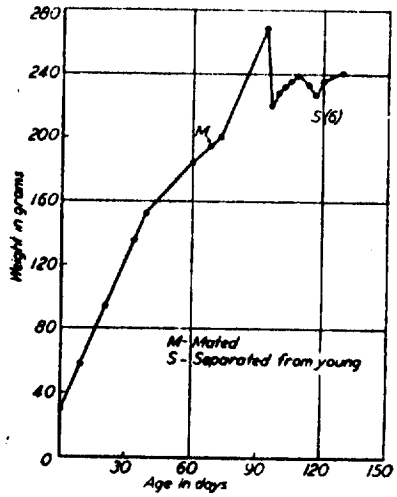

FIG. 4

μg./day, *p*-aminobenzoic acid plus inositol, each 0.5 mg./day, and yeast nucleic acid, 30 mg./day.

Brewers' yeast[2] when fed to rats as a supplement (500 mg./day) to the purified ration prevented the loss in weight of lactating mothers.[3] (Fig. 4).

[2] Brewers' yeast powder, strain K. We are indebted to Anheuser-Busch, Inc., St. Louis, Mo., for a generous supply of this material.

[3] This loss in weight of lactating mothers was also prevented in the case of mice kept on highly purified diets, by a supplement of a folic acid concentrate (19). Preliminary experiments on rats, using this concentrate have shown an improved average weaning weight of litters and only a slight loss in the weight of lactating mothers.

Discussion

The use of several highly purified diets in which the content and type of fat differed was planned because of certain results that were obtained with mice in this laboratory. When Crisco was the sole source of fat in the diet, mice failed to reproduce. A suspicion that the essential fatty acids were present in insufficient quantity in this fat arose when several cases of resorption occurred. On addition of either lard or corn oil to the diet, resorptions disappeared and successful lactation was obtained. These two fats are known to be rich sources of linoleic acid. Apparently, Rogers, *et al.* (14), in raising mice through two generations on an artificial diet, encountered a similar deficiency in essential fatty acids since in addition to Crisco, their ration contained a supplement of linoleic acid. However, in the past year we have found that it is possible to raise litters on diets containing Crisco as the only fat (diet R-6). Whether there has been a change in the preparation of Crisco or the early work with mice was inconclusive has not been determined.

Throughout the four generations the rats on the purified diets compared favorably with control animals in every respect except during lactation. Here we have observed the sharp loss in weight of the mothers. These rats exert themselves to the utmost in supplying nourishment to their young. Since the diet apparently fails to supply some factor in sufficient quantity to satisfy the demands of lactation, the mother in an effort to continue maximum milk production breaks down her own body tissue to supply the missing substance necessary for adequate lactation. This idea is substantiated by the observation that rats nursing small litters lost less weight than mothers taking care of large litters. When the tissue breakdown reaches a critical stage, the mother either succumbs or the litter is destroyed. The several cases of coprophagy observed in the case of the black rats are highly significant since they point to a nutritional deficiency while under the strain of lactation.

The idea of the existence of a specific lactation factor, a substance that initiates the lactation mechanism and without which no milk can be produced by the mother, has been put forward by Mapson (15), and by Nakahara, *et al.* (16, 17). The former was unable to obtain successful lactation in rats on a purified diet containing yeast as a source of the vitamin B complex. Approximately 70% of the young born died within 3 days after parturition. Upon feeding whole liver or an aqueous extract of it, normal lactation was obtained. Mapson gave the name "physin"

to the substance in liver responsible for this effect. Nakahara also assumes the existence of specific lactation factors, and in a series of papers has attempted to demonstrate the necessity of two such substances.

The results of our experiments do not lend support to the idea of a true lactation factor since it was possible to wean a moderately high percentage of litters through several generations on highly purified rations. What they indicate, however, is that a lactagogue, some substance causing increased milk production, is lacking in the purified diets. Such a substance seems to be present in yeast.

Summary

1. Several highly purified rations have been employed in raising rats belonging to two different strains through four generations.

2. Growth on these diets was excellent throughout the four generations and compared favorably with that of controls kept on Purina dog chow.

3. Invariably, the mothers lost weight during lactation which was regained as soon as they were separated from the young.

4. Of the several supplements used to prevent the loss in weight during lactation only brewers' yeast was found to be beneficial.

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Effect of saturated and unsaturated fats on hepatic synthesis and biliary excretion of cholesterol by the rat[1]

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WILSON, JEAN D. AND MARVIN D. SIPERSTEIN. *Effect of saturated and unsaturated fats on hepatic synthesis and biliary excretion of cholesterol by the rat.* Am. J. Physiol. 196(3): 599-602. 1959. In an attempt to elucidate the mechanisms by which saturated and unsaturated fats influence cholesterol metabolism, cholesterol synthesis in rat liver slices and the excretion of cholesterol end products into the bile of rats was studied during variations in the type and quantity of dietary fat. Neither 30% lard nor 30% corn oil diets had any effect on either the excretion of total cholesterol-C^{14} end products or of cholesterol itself into bile. Furthermore, the feeding of lard or corn oil, at either the 10% or 30% level, had no effect on the rate of conversion of acetate or mevalonate to cholesterol. These studies are interpreted as suggesting that the effects of saturated and unsaturated fats on cholesterol levels in rats are mediated at some phase of cholesterol metabolism other than the hepatic synthesis or hepatic degradation and excretion of this sterol.

DURING THE PAST SEVERAL YEARS considerable evidence has accrued indicating that in both man (2-10) and certain experimental animals (11) the level of serum cholesterol is at least partially dependent upon the type and quantity of dietary fat; in rats, however, alterations in cholesterol metabolism with variations in dietary fat are primarily reflected by changes in liver cholesterol concentration (12-14). Two general concepts have emerged from these observations: *a*) the intake of large amounts of saturated fat appears to be associated with elevations of cholesterol, and *b*) the intake of certain unsaturated fats is often followed by a lowering of cholesterol concentration below the levels achieved with fat-free diets. Although biochemical studies (15-18) have attempted to identify the substance or substances in fats responsible for these effects, the specific mechanisms by which either saturated or unsaturated fats influence cholesterol metabolism are poorly understood.

The present series of experiments was undertaken in an attempt to identify the manner in which variations in the intake of dietary fat might influence cholesterol metabolism. This relationship was examined in the rat by studying both cholesterol synthesis in rat liver slices and the excretion of cholesterol end-products into bile during variations in the type and quantity of fat intake.

METHODS AND PROCEDURE

Male rats of the Long-Evans strain, weighing 150-350 gm, were maintained on Purina laboratory chow until the experiments were begun. Experimental diets were then fed for periods varying from 1 to 16 days. The experimental diets consisted of two different fat-free synthetic mixtures[2] to which varying amounts of lard or corn oil were added. During the 1-day experiments the diets were homogenized with water in a Waring Blendor and were intubated into unanesthetized rats by stomach tube. In all other experiments the animals were pair-fed isocaloric quantities of the solid diet. Water was allowed ad libitum throughout the experiment.

Cholesterol excretion studies. Following the initial periods (1-16 days) on the experimental diets, the rats used for the bile excretion studies were anesthetized with ethyl ether, and cannulae were inserted into their common bile ducts as previously described (19). A solution of cho-

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[1] This investigation was aided in part by grants from the American Heart Association and the Dallas Heart Association. A report of these studies in abstract form has been published (1).

[2] For experiments in which up to 10% fat was fed, lard or corn oil was added to a biological test diet supplied by General Biochemicals, Inc.; it contained 18% casein, 74% glucose, 4% brewers' yeast, 4% USP XIV salt mix, and added α-tocopherol, Ca pantothenate, choline, linoleic acid, pyridoxine, riboflavin, vitamin A and vitamin D. When greater amounts of lard or corn oil were fed, the fat was added to a second test diet, each 1000 gm of which contained 67% glucose, 26% casein, 7% Hawk Oser salt mix, 0.5 gm inositol, 1 bottle Folbesyn (Lederle), 10 mg folic acid and 0.1 ml Oleum Percomorphum (Mead Johnson). When the diet contained 30% fat or above, the vitamin content was adjusted so that the lard and corn oil diets contained the same amount of vitamins as the fat-free diet.

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TABLE 1. *Influence of Saturated and Unsaturated Fats on Biliary Excretion of Cholesterol End Products*

Rat No.	Fat Content of Diet	Duration of Feeding, Days	Cumulative Percentage of Administered Dose Recovered in Bile as: Total cholesterol-4-C^{14}-end products						Cholesterol-4-C^{14}	Total Cholesterol Excreted in Bile, mg/48 hr.
			2 Hr.	6 Hr.	12 Hr.	24 Hr.	36 Hr.	48 Hr.	48 Hr.	
Experiment 1										
1	None	1	5.19	12.48	19.82	30.26	37.58	41.38	2.47	2.66
2	None	1	5.15	11.61	15.96	22.82	31.38	38.87	2.70	3.44
3	None	1	5.94	15.95	23.33	36.05	49.46	53.45	3.82	2.91
4	None	1	3.38	9.51	14.94	25.18	36.10	47.41	3.08	3.75
5	30% Corn oil	1	3.80	11.90	17.35	27.71	35.61	38.23	2.13	
6	30% Lard	1	2.46	10.66	21.29	33.71	42.27	46.80	4.93	5.74
Experiment 2										
7	None	6	5.19	9.38	17.98	38.04	51.14	60.99	3.76	4.63
8	None	6	1.69	3.75	5.64	11.97	23.86	32.37	3.13	6.02
9	30% Corn oil	6	4.89	11.20	17.35	35.06	44.77	53.38	3.51	4.63
10	30% Lard	6	5.19	10.71	16.15	29.84	42.61	56.05	2.96	5.19
Experiment 3										
11	None	7	5.20	9.74	19.10	27.75	39.10	50.70		3.82
12	None	7	5.25	11.23	17.02	43.52	67.42	79.29		4.95
13	None	7	9.76	17.41	26.58	39.87	49.66	56.78		3.80
14	30% Corn oil	7	5.28	13.40	22.88	39.34	55.74	67.59		4.57
15	30% Corn oil	7	5.96	14.38	21.55	34.02	47.96	54.42		4.82
16	30% Lard	7	4.06	10.31	15.55	25.42	36.34	43.88		3.05
17	30% Lard	7	3.48	9.32	16.81	24.89	34.58	41.72		3.03
Experiment 4										
18	None	16	0.27	10.45	15.86	32.36	44.06	52.72	1.54	1.69
19	None	16	5.18	12.63	19.14	36.61	46.86	53.97	2.99	1.57
20	None	16	1.62	8.62	12.85	26.46	38.07	43.33	2.21	1.63
21	30% Corn oil	16	5.19	12.02	17.51	32.08	45.81	51.14	1.61	1.66
22	30% Corn oil	16	5.06	12.06	17.18	29.91	40.11	47.34	3.26	1.82
23	30% Corn oil	16	9.98	18.86	24.97	47.26	63.35	68.50	2.97	4.02
24	30% Lard	16	4.67	10.08	15.11	25.15	35.35	40.36	1.82	1.52
25	30% Lard	16	3.80	9.08	13.07	25.32	36.73	46.30	1.52	1.45
26	30% Lard	16	1.32	7.10	11.63	23.03	35.18	44.99	1.57	1.59

lesterol-4-C^{14}, prepared with the aid of Tween 20 (20) and containing 2.75×10^5 cpm, was then injected into the inferior vena cava, and the rats were placed in restraining cages. Bile was collected in tared vials at intervals for 48 hours; during the periods of bile collection food was allowed ad libitum, and the rats were given drinking water containing 0.1% KCl. Two-tenths-milliliter aliquots of the bile were mounted in 16 ml of 0.4% diphenyloxazole in toluene-ethyl alcohol (3:1), and the amount of C^{14} was determined in a liquid scintillation counter. The cholesterol content of bile was determined by the method of Sperry and Webb (21); in addition an aliquot of the digitonide was dissolved in methanol, added to 16 ml of 0.4% diphenyloxazole in toluene, and assayed for C^{14} content.

Cholesterol synthesis studies. The rats used for the studies of cholesterol synthesis were killed by a blow on the head, and their livers were quickly excised, weighed and placed in ice-cold Krebs-Ringer-bicarbonate buffer, pH 7.4. Slices, approximately 0.5 mm thick, were prepared free hand; 500-mg portions of slices were placed in 50-ml Erlenmeyer flasks containing glass center wells. Each flask contained 5 ml of either Krebs-Ringer-bicarbonate or Krebs-Ringer-phosphate buffer, pH 7.4, and either 10.1 μmoles of potassium acetate-C^{14} containing 4.75×10^5 cpm or 0.6 μmoles of potassium mevalonate-2-C^{14} containing 5.71×10^5 cpm. The flasks were gassed with 95% O_2-5% CO_2, stoppered with serum caps and incubated at 37.5° in a Dubnoff metabolic shaker for 60 minutes.

At the end of the incubation period the reaction was stopped by the injection of 0.25 ml 10 N H_2SO_4 into the outer chamber of the flask. Carbon dioxide was trapped by a modification of the procedure described by Passmann, Radin and Cooper (22); ½ ml 1 M Hyamine hydroxide in methanol was injected into the center well, and the flasks were shaken at 0°C for 30 minutes, following which the Hyamine-CO_2 was transferred with toluene into 10-ml volumetric flasks. One-milliliter aliquots of the toluene-Hyamine-CO_2 solution were mounted in 16 ml of 0.4% diphenyloxazole in toluene and assayed for C^{14}.

To the outer chamber of the flasks was then added 1 ml of 90% KOH, and the contents were saponified at 15 pounds pressure and 120°C for 30 minutes. The contents of the flasks were then transferred with water and ethyl alcohol into 250-ml Erlenmeyer flasks and extracted twice with approximately 150 ml of petroleum ether. The petroleum ether fraction was then evaporated to dryness, dissolved in warm acetone-ethyl alcohol (1:1,

TABLE 2. *Influence of Diets Containing 10% Fat on Cholesterol Synthesis From Acetate-1-C^{14} in Rat Liver Slices*

Rat No.	Fat Content of Diet	Duration of Feeding, Days	Rat Weight/Liver Weight, gm	Percentage of Administered Dose Recovered as:	
				Cholesterol	CO_2
Experiment 1					
1	None	1	214/8.0	1.49	34.07
2	None	1	219/9.2	0.51	37.85
3	None	1	229/9.4	0.85	59.81
4	10% Corn oil	1	242/11.8	1.29	37.83
5	10% Corn oil	1	207/9.0	1.41	43.56
6	10% Corn oil	1	212/9.1	1.80	59.22
7	10% Lard	1	190/9.0	0.86	50.15
8	10% Lard	1	191/8.8	1.36	69.61
Experiment 2					
9	None	6	137/6.8	0.06	11.62
10	None	6	167/8.4	0.35	18.52
11	None	6	178/7.4	0.44	36.72
12	10% Corn oil	6	160/7.9	0.28	26.45
13	10% Corn oil	6	168/9.4	0.22	23.90
14	10% Corn oil	6	182/9.9	0.82	33.43
15	10% Lard	6	156/7.9	0.12	22.48
16	10% Lard	6	186/9.4	0.13	28.68

TABLE 3. *Influence of Diets Containing 30% Fat on Cholesterol Synthesis From Acetate-1-C^{14} or Mevalonate-2-C^{14} in Rat Liver Slices*

Rat No.	Fat Content of Diet	Duration of Feeding, Days	Rat Weight/Liver Weight, gm	Percentage of Administered Dose Recovered From			
				Mevalonate-2-C^{14} as:		Acetate-1-C^{14} as:	
				Cholesterol	CO_2	Cholesterol	CO_2
1	None	5	181/7.5	2.21	0.72	0.27	40.88
2	None	5	164/6.9	2.82	0.72	0.03	53.66
3	None	5	207/8.2	2.91	0.79	0.09	62.73
4	30% Corn oil	5	142/6.7	2.21	0.65	0.36	34.58
5	30% Corn oil	5	188/9.7	3.04	0.77	0.56	31.79
6	30% Corn oil	5	184/8.1	3.01	0.76	0.33	40.82
7	30% Lard	5	174/7.6	2.59	0.68	0.22	52.30
8	30% Lard	5	162/6.4	2.01	0.59	0.07	28.48
9	30% Lard	5	190/7.4	2.44	0.73	0.16	51.54

[illegible] acidified with 1 N HCl to pH 3.0. Cholesterol digi[illegible]de was precipitated and washed as described by [illegible]rry and Webb (21) and dissolved in methyl alcohol. [illegible] aliquot of this solution was added directly to 16 ml [illegible] diphenyloxazole in toluene and assayed for C^{14}.

RESULTS

Biliary excretion of cholesterol and bile acids. The results [illegible]udies of the influence of saturated and unsaturated [illegible] on the excretion of cholesterol end-products are [illegible]n in table 1. Four separate experiments involving [illegible]ats were performed in an attempt to show an influ[illegible] of fat feeding for periods of 1, 6, 7 and 16 days on [illegible] elimination of cholesterol into bile. During the 48-[illegible] period of bile collection, a significant percentage of [illegible] administered cholesterol (32-79%) was excreted into [illegible]bile. However, the fat content of the diet did not influence either the biliary excretion of cholesterol-C^{14} or of total cholesterol. Moreover, the rate of cholesterol degradation, as measured by the excretion of total cholesterol-C^{14} end products, was uninfluenced by the feeding of corn oil or lard for periods of as long as 16 days.

Synthesis of cholesterol by liver slices. Two sets of experiments were performed in an attempt to demonstrate an effect of fat feeding on cholesterol synthesis in rat liver slices. In one group of experiments, shown in table 2, the effect of the feeding of diets containing either 10% lard or 10% corn oil was studied. The feeding of 10% fat for as long as 6 days had no apparent effect on the rate of cholesterol synthesis from acetate or on the rate of conversion of acetate to CO_2.

To determine whether the rate of conversion of acetate under these circumstances was in fact a valid measure of cholesterol synthesis, diets containing 30% corn oil or 30% lard were fed for 5 days, after which the conversion of both mevalonate-2-C^{14} and acetate-1-C^{14} to cholesterol was determined in rat liver slices (table 3). Once again, the fat diets had no effect on either the formation of cholesterol or of CO_2 from these two precursors.

DISCUSSION

The level of cholesterol within any body compartment can be regarded as the net result of the various reactions involved in cholesterol metabolism, namely cholesterol absorption, cholesterol synthesis, cholesterol transport, cholesterol excretion and the degradation of cholesterol. The effects of feeding saturated or unsaturated fats on three aspects of cholesterol metabolism in the rat—cholesterol excretion into the bile, the degradation of cholesterol by the liver, and cholesterol synthesis by the liver—are reported here.

No significant effect on either the degradation or biliary excretion of cholesterol could be detected in these experiments following the feeding of diets containing either 30% lard or corn oil for periods varying from 1 to 16 days. It seems likely, therefore, that the influence of fats on sterol levels in the rat is not mediated by an effect on these two aspects of cholesterol metabolism.

Since a major excretory product of cholesterol consists of bile acids, the finding of Lewis (23) and of Gordon *et al.* (24) that bile acid excretion was markedly increased in both the bile and feces of human subjects fed sunflower seed oil as compared to isocaloric amounts of hydrogenated coconut oil has suggested that the effect of unsaturated fats on cholesterol metabolism may be secondary to increased cholesterol destruction. In the present series of experiments in rats both the degradation of cholesterol to bile acids and the excretion of biliary cholesterol were normal—indicating that two of the major pathways for cholesterol excretion remain intact despite variations in the type and quantity of dietary fat. The reasons for the apparent discrepancy between our findings and those in human beings are not apparent and may represent a species variation.

The second group of experiments reported here was

undertaken in an attempt to elucidate the relationship of dietary fat to cholesterol synthesis. It is clear from these experiments that the pair-feeding of isocaloric quantities of diets containing no fat, 10% lard or 10% corn oil has no influence on the conversion of acetate to cholesterol *in vitro*. However, since Curran (25) has shown that acetoacetate may serve as a cholesterol precursor without first being degraded to acetyl-CoA, it was possible that in the presence of a large amount of dietary fat the acetoacetyl-CoA pool might become so large that acetate incorporation into cholesterol would no longer be a valid measure of cholesterol synthesis. It has now been clearly established that mevalonate is a more immediate precursor of cholesterol than is acetyl-CoA (26), and the incorporation of mevalonate into cholesterol should, therefore, give a more valid measure of cholesterol synthesis than would acetate incorporation. Since the conversion of both acetate and mevalonate to cholesterol is unaffected by the feeding of 30% lard or corn oil, the conclusion may be drawn that in pair-fed rats, dietary fat has no effect on the hepatic synthesis of cholesterol *in vitro*.

The conclusion that in the rat the influence of saturated and unsaturated fats on cholesterol metabolism is probably not mediated through the hepatic synthesis or excretion or destruction of this sterol suggests that saturated and unsaturated fatty acids exert their effects at other stages of sterol metabolism. Likely sites which have not yet been studied include cholesterol transport, cholesterol esterification and the intestinal excretion and absorption of cholesterol.

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