MONOGRAPH

ON

HYDROCHLORIC ACID

Monograph on **Hydrochloric Acid** #92 7/26/74

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TR-72-1552-50

Submitted Under:
Contract No. FDA 72-104

July 26, 1974

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HYDROCHLORIC ACID

Summary

Because of its metabolic functions and its widespread use in industry, hydrochloric acid has been the subject of considerable research.

Shay et al. (419) reported that hydrochloric acid is not absorbed by the human stomach. Upon passing through the normally acid (pH 1.5-2.5) stomach, the hydrochloric acid is neutralized by alkaline secretions in the intestines (209, 423).

If more acid is ingested than can be readily neutralized, excess acid or acid products may be absorbed, resulting in metabolic acidosis. When this occurs, neutralization is immediately effected by buffer salts and the alkali bicarbonate of the plasma. This results in a reduction of the bicarbonate-carbonic acid ratio and pH of the blood. In an attempt to restore normalcy, hyperventilation and renal retention of bicarbonate occur (138, 452).

A mild metabolic acidosis may be asymptomatic. Symptoms usually appear when the CO_2 content falls to 18.2 mEq/l, or lower. The patient may complain of weakness or general malaise, or dull headache. In addition, nausea, vomiting, and abdominal pain may be present. Characteristic very deep respirations are often present. The respiratory rate is also usually increased, but may be slow. These signs are usually present when the CO_2 content falls below 9.1 mEq/l, and the pH is 7.2 or lower. When the pH falls to 7.0, respiratory depression may occur. Examination of the electrocardiogram often reveals signs of hypopotassemia. Severe acidosis can also cause RS-T deviations of the type usually found in myocardial injury. An excess elimination of water and neutral salts by the kidneys may lead to dehydration and possibly to some loss in vital activity of the tissues. A leukocytosis may be present. The urine of acidotic subjects has a high ammonia content and is very acid (138, 452).

Through an extensive series of experiments, Fazekas (111, 113, 114) found that the development of acidosis through the continued administration of hydrochloric acid can cause the following results in rabbits: hypertrophy and increased function of the adrenal cortex; enlargement of the ovaries and uterus; follicle maturity and follicle hemorrhage; corpora lutea formation; cyclic changes of the mucosa of the uterine horns; enlargement of the breasts and milk secretion; enlargement and increased function of the parathyroids; enlargement of the pituitary gland and multiplication of the basophilic cells of the anterior lobe of the pituitary.

Upon maintaining a child of 6 for 6 days on an exclusive diet of milk containing 0.05 N hydrochloric acid, Telfer (452) observed an increased renal output of CaO, MgO and P_2O_5, indicating increased absorption of Ca,

Mg and P. Decreased retention of these minerals was also observed. The author suggested that these results indicated that hydrochloric acid adversely affects the process of calcification. Code et al. (71) reported that gastric intubation of hydrochloric acid in dogs inhibited gastric absorption of Na but had no effect on the absorption of water and K. Upon oral administration of 3.5 g hydrochloric acid daily for 3 days to human subjects, Stehle and McCarty (430) observed increased urinary excretion of K, Na, NH_3, H_3PO_4, and H ions.

Deloyers and Suprez (84) reported that intraduodenal hydrochloric acid exhibits a strong hypoglycemic effect in man. Boattini (40), however, disputed this finding.

Normal hydrochloric acid secretion into the stomach is proportionally depressed by introduction of the acid into the stomach or duodenum (11, 203). Pepsin secretion was found by Johnson (201) to be enhanced by irrigation of the gastric mucosa with hydrochloric acid.

A number of researchers have reported that the addition of hydrochloric acid to test meals inhibits gastric motility and slows gastric emptying (179, 329), while other workers have stated that the acidity of the contents of the stomach and duodenum has no effect on the emptying time of the stomach (47, 277, 279).

MacGilvra and Ellison (104, 263) reported that small amounts of intravenously administered hydrochloric acid were effective in accelerating termination of ether and avertin anesthesia. Other workers, however, were unable to confirm these results, and, in addition, reported that the acid was ineffective in terminating anesthesia from nembutal or paraldehyde (216, 415, 416).

In experiments performed on cats and dogs, Chandler and Chopra (67) observed that hydrochloric acid increased the toxicity of CCl_4 when both were administered orally.

A fair amount of research has been conducted on the toxicological aspects of hydrochloric acid. The carcinogenicity of the acid has been tested by two teams of researchers. Suntzeff et al. (439) reported the formation of spindle-cell sarcomas in 4 of 8 mice at or near the site of injection following s.c. administration of 0.5% hydrochloric acid (0.25 ml daily, six days per week, up to 16 months). However, inadequate controls were maintained, so the results were not conclusive. No tumors were induced by Dyer et al. (99) by the administration (esophageal intubation) of up to 92 doses (0.01 ml/g body weight) of 0.25-1.0 N hydrochloric acid.

On the basis of experiments in which White Leghorn eggs were injected with hydrochloric acid prior to incubation, Landauer (241) reported that concentrated hydrochloric acid probably produces a significant rise in the incidence of rumplessness; dilute acid (pH about 1) had no effect. The intraamniotic injection of 17 mouse fetuses with 2 microliters 0.1 M hydrochloric acid on the 13th day of gestation was found by Dostal (91) to produce no teratogenic effects; fetal mortality, however, was slightly (5.8%) increased by the acid.

Following tests on 228 patients, Hardy (154) concluded that hydrochloric acid plays no essential part in ordinary gastric pain production.

Matzner (274) reported that 0.3% hydrochloric acid, when administered alone to non-fasting rats, is relatively ineffective in the production of gastric ulcers.

Two teams of workers have tested the toxicity of hydrogen chloride gas. An inhalation study by Higgins et al. (168) established an LC_{50} of 40,989 ppm for rats and 13,745 ppm for mice with a 5 minute exposure time. In the experiments of Machle et al. (264), performed on rabbits, guinea pigs, and monkeys, a concentration of 6.5 mg/l resulted in 100% fatality in 30 minutes and 1 mg/l gave the same results if the exposure was of 2-6 hours. Machle et al. (264) found that the effect of exposure on the growth of the animals was not remarkable; a sharp, but temporary loss of weight was evident in every instance.

A number of case studies of fatal overdoses of hydrochloric acid have been reported. In general, when concentrated hydrochloric acid is swallowed it passes rapidly through the esophagus into the stomach, whereupon it induces pyloric contraction and is trapped in the distal end of the stomach. It is at this point that it does its greatest damage, causing extensive necrosis before being neutralized. If it perforates the stomach death ensues rapidly; otherwise the patient will begin to feel better after several days and apparently recover. However, 3-4 weeks after ingestion, pyloric stenosis develops and may lead to death unless corrected surgically. The severity of damage produced by acid ingestion varies with the quantity and concentration of acid consumed and the state of the stomach contents at the time of consumption (207, 462, 243, 325, 29, 21, 137, 347, 431).

In the report of a suicide case, Velten (471) attributed death to a cause not mentioned by other authors. He suggested that the subject succumbed as a result of "an absorptive protein autointoxication" caused by the absorption of protein decomposition products produced in the acid scaling of the mucosa.

The acute toxicity of hydrochloric acid has received little study. In tests by Hermann et al. (164), the largest non-fatal amount of 0.25 N acid that was tolerated by 2 kg rabbits was 0.6 g/kg. On the basis of reported cases of death from overdose with hydrochloric acid, Poison and Tattersall (341) suggested that the lethal dose of hydrochloric acid for man is probably of the order of an ounce or less.

Although no long-term studies have been found in the available literature, several short-term studies have been reported. When fed to chicks for a period of 27 days, Pritzl and Kienholz (351) found that 0.21 and 0.42% hydrochloric acid produced no significant effects. However, a concentration of 0.84% resulted in a lower body weight gain and feed efficiency. In a 9-week study by Burns (52), rats were given hydrochloric acid in doses that gradually increased from the initial 12 ml of 0.1 N acid per day until at the end of 7 weeks they were each receiving 50 ml of 0.1 N acid daily; for the last 2 weeks they were given 50 ml of 0.2 N hydrochloric acid daily. The treated rats died after 9 weeks, where-

upon it was determined that both bone and body growth had been slightly retarded by the consumption of the acid. In 180 g rats given, intraperitoneally, a total of 6 doses of 3 or 6 mg hydrochloric acid over a period of 4 days, Beznak et al. (38) observed a considerable hypertrophy of the adrenals. In a study by Burns (52), rabbits were given 1 N hydrochloric acid in varying amounts up to 200 ml over a period of 35 days. These animals showed either retarded growth or actual weight loss, severe malnutrition, reduction in the base content of the muscles, and a marked reduction in the fat content of the bones.

HYDROCHLORIC ACID

Chemical Information

I. Nomenclature

A. Common Names

1. Hydrochloric acid
2. Muriatic acid
3. Chlorohydric acid

B. Chemical Names

1. Hydrochloric acid

C. Trade Names

None

D. Chemical Abstracts Registry Number

Hydrochloric acid 007647-01-0

Hydrochloric acid, diluted 977000-05-7

II. Empirical Formula

HCl

III. Structural Formula

HCl

IV. Molecular Weight

36.46

V. Specifications

Food Chemicals Codex

Assay	Not less than the minimum or within the range of Baume degrees claimed or implied by the vendor
Color	Passes test
Concentration of HCl	Not less than the minimum or within the range specified or implied by the vendor

Specific gravity	Not less than the minimum or within the range specified or implied by the vendor
Arsenic (as As)	Not more than 1 ppm (0.0001%)
Heavy metals (as Pb)	Not more than 5 ppm (0.0005%)
Iron	Not more than 5 ppm (0.0005%)
Nonvolatile residue	Not more than 0.5%
Oxidizing substances (as Cl_2).	Not more than 30 ppm (0.003%)
Reducing substances (as SO_3)	Not more than 70 ppm (0.007%)
Sulfate	Not more than 0.5%

VI. Description

A. General Characteristics

Hydrochloric acid is an aqueous solution of hydrogen chloride of varying concentrations. It is a colorless to slightly yellow liquid having a sharp, pungent odor.

B. Physical Properties

Concentrations of hydrochloric acid commercially available are usually expressed in Baume degrees (Be') from which percentages of HCl and specific gravities can readily be derived (see Table 1). Concentrations above 8.5 Be' (12.5%) fume in moist air, lose hydrogen chloride, and create a corrosive atmosphere. The pH of 1.0 N HCl is 0.10, of 0.1 N = 1.10, of 0.01 N = 2.02, of 0.001 N = 3.02, of 0.0001 N = 4.01. All aqueous solutions of HCl, on boiling, approach a constant boiling mixture containing 20.24% HCl (approx. 13.4 Be') which boils at 110 degrees C (760 mm Hg). The melting point of 18 Be' HCl is -42 degrees C, of 20 Be' is -53 degrees C, of 22 Be' is -66 degrees C, of 23 Be' is -74 degrees C. Additional information is presented in Tables 2-8.

TABLE 1.

Be.°	Sp. Gr.	% HCl	Be.°	Sp. Gr.	% HCl
1.00	1.0069	1.40	16.6	1.1292	25.56
2.00	1.0140	2.82	16.7	1.1301	25.72
3.00	1.0211	4.25	16.8	1.1310	25.89
4.00	1.0284	5.69	16.9	1.1319	26.05
5.00	1.0357	7.15	17.0	1.1328	26.22
5.25	1.0375	7.52	17.1	1.1336	26.39
5.50	1.0394	7.89	17.2	1.1345	26.56
5.75	1.0413	8.26	17.3	1.1354	26.73
6.00	1.0432	8.64	17.4	1.1363	26.90
6.25	1.0450	9.02	17.5	1.1372	27.07
6.50	1.0469	9.40	17.6	1.1381	27.24
6.75	1.0488	9.78	17.7	1.1390	27.41
7.00	1.0507	10.17	17.8	1.1399	27.58
7.25	1.0526	10.55	17.9	1.1408	27.75
7.50	1.0545	10.94	18.0	1.1417	27.92
7.75	1.0564	11.32	18.1	1.1426	28.09
8.00	1.0584	11.71	18.2	1.1435	28.26
8.25	1.0603	12.09	18.3	1.1444	28.44
8.50	1.0623	12.48	18.4	1.1453	28.61
8.75	1.0642	12.87	18.5	1.1462	28.78
9.00	1.0662	13.26	18.6	1.1471	28.95
9.25	1.0681	13.65	18.7	1.1480	29.13
9.50	1.0701	14.04	18.8	1.1489	29.30
9.75	1.0721	14.43	18.9	1.1498	29.48
10.00	1.0741	14.83	19.0	1.1508	29.65
10.25	1.0761	15.22	19.1	1.1517	29.83
10.50	1.0781	15.62	19.2	1.1526	30.00
10.75	1.0801	16.01	19.3	1.1535	30.18
11.00	1.0821	16.41	19.4	1.1544	30.35
11.25	1.0841	16.81	19.5	1.1554	30.53
11.50	1.0861	17.21	19.6	1.1563	30.71
11.75	1.0881	17.61	19.7	1.1572	30.90
12.00	1.0902	18.01	19.8	1.1581	31.08
12.25	1.0922	18.41	19.9	1.1590	31.27
12.50	1.0943	18.82	20.0	1.1600	31.45
12.75	1.0964	19.22	20.1	1.1609	31.64
13.00	1.0985	19.63	20.2	1.1619	31.82
13.25	1.1006	20.04	20.3	1.1628	32.01
13.50	1.1027	20.45	20.4	1.1637	32.19
13.75	1.1048	20.86	20.5	1.1647	32.38
14.00	1.1069	21.27	20.6	1.1656	32.56
14.25	1.1090	21.68	20.7	1.1666	32.75
14.50	1.1111	22.09	20.8	1.1675	32.93
14.75	1.1132	22.50	20.9	1.1684	33.12
15.00	1.1154	22.92	21.0	1.1694	33.31
15.25	1.1176	23.33	21.1	1.1703	33.50
15.50	1.1197	23.75	21.2	1.1713	33.69
15.75	1.1219	24.16	21.3	1.1722	33.88
16.0	1.1240	24.57	21.4	1.1732	34.07
16.1	1.1248	24.73	21.5	1.1741	34.26
16.2	1.1256	24.90	21.6	1.1751	34.45
16.3	1.1265	25.06	21.7	1.1760	34.64
16.4	1.1274	25.23	21.8	1.1770	34.83
16.5	1.1283	25.39	21.9	1.1779	35.02
22.0	1.1789	35.21	23.8	1.1963	38.95
22.1	1.1798	35.40	23.9	1.1973	39.18
22.2	1.1808	35.59	24.0	1.1983	39.41
22.3	1.1817	35.78	24.1	1.1993	39.64
22.4	1.1827	35.97	24.2	1.2003	39.86
22.5	1.1836	36.16	24.3	1.2013	40.09
22.6	1.1846	36.35	24.4	1.2023	40.32
22.7	1.1856	36.54	24.5	1.2033	40.55
22.8	1.1866	36.73	24.6	1.2043	40.78
22.9	1.1875	36.93	24.7	1.2053	41.01
23.0	1.1885	37.14	24.8	1.2063	41.24
23.1	1.1895	37.36	24.9	1.2073	41.48
23.2	1.1904	37.58	25.0	1.2083	41.72
23.3	1.1914	37.80	25.1	1.2093	41.99
23.4	1.1924	38.03	25.2	1.2103	42.30
23.5	1.1934	38.26	25.3	1.2114	42.64
23.6	1.1944	38.49	25.4	1.2124	43.01
23.7	1.1953	38.72	25.5	1.2134	43.40

TABLE 2. Physical and Chemical Properties of Hydrogen Chloride

boiling point, °C at 760 mm Hg	−85.034
freezing point, °C at saturation pressure (triple point)	−114.19
density of gas, g/ml	
at 20°C	0.001526
at 25°C	0.001500
refractive index of gas	
n_D^{20} at 1 atm	0.000415
n_D^{25} at 1 atm	0.000408
viscosity of gas, cP	
at 0°C	0.01379
at 20°C	0.01560
at 100°C	0.01822
viscosity of 1.0 *M* solution, cP at 25°C	0.95366
surface tension, dyn/cm, at −92.9°C	24.718
specific heat, gas, cal/(g)(°C)	
at constant pressure, C_p, at 15°C	0.1939
at constant volume, C_v, at 15°C	0.1375
heat of fusion, cal/g-mole (crystal form I to liquid at −114.22°C)	476.
heat of vaporization, cal/g-mole at −85.05°C	3860
heat of solution to infinite dilution, kcal/g-mole	17.89
critical temperature, °C	51.40
critical pressure, atm	81.5
critical density, g/ml	0.42
enthalpy of formation at 25°C, kcal/g-mole	
of gas	−22.062
of aqueous solution (500 moles H_2O/mole HCl)	−39.812
Gibbs free energy of formation at 25°C, kcal/g-mole	
of gas	−22.774
of HCl in aqueous solution, at std. state, m = 1	−31.372
electrical conductivity of liquid at −96°C, mho/cm	1×10^{-8}
dipole moment, D, at −71 to 316°C	1.05 ± 0.004
dielectric constant of gas at 0°C and 760 mm Hg	1.0046

TABLE 3. Solubility of Hydrogen Chloride in Water at 760 mm Hg

temperature, °C	0	30	40	50	60
solubility, g HCl/100 g H_2O	82.31	67.30	63.07	59.59	56.10

TABLE 4. Specific Gravity of Aqueous Hydrochloric Acid Solutions

°Baumé	5.00	10.00	15.00	18.00	20.00	22.00	23.00	25.00
d_{60}^{60}	1.0357	1.0741	1.1154	1.1417	1.1600	1.1789	1.1885	1.2083
hydrogen chloride, wt %	7.15	14.83	22.92	27.92	31.45	35.21	37.14	41.72

TABLE 5. Specific Heats of Aqueous Hydrochloric Acid Solutions

Temperature, °C	Specific heat, cal/(g)(°C), of hydrogen chloride, wt %			
	16.8	28.9	33.6	41.4
0	0.72	0.61	0.58	0.55
20	0.74	0.631	0.591	
60	0.78	0.67	0.638	0.61

TABLE 6. Partial Pressure of Hydrogen Chloride over Aqueous Solutions of Hydrochloric Acid

Hydrogen chloride, wt %	Partial pressure, mm Hg, at					
	0°C	10°C	30°C	60°C	100°C	110°C
6	0.00007	0.0002	0.002	0.04	0.92	1.78
10	0.0004	0.001	0.01	0.16	2.9	5.4
20	0.03	0.08	0.48	4.4	49.0	83.0
30	2.4	5.23	21.0	124.0	845.0	
40	130.0	233.0	627.0			

TABLE 7. Partial Pressures of Water over Aqueous Solutions of Hydrochloric Acid

Hydrogen chloride, wt %	Partial pressure, mm Hg, at					
	0°C	10°C	30°C	60°C	100°C	110°C
6	4.18	8.45	29.1	139	715	
10	3.84	7.70	26.8	130	677	960
20	2.62	5.40	19.0	93.5	510	729
30	1.26	2.73	10.2	53.5	310	444
40	0.41	0.94	4.09	25.0	158	230

TABLE 8. Solubility of Hydrogen Chloride in Organic Solvents

Solvent	Solubility, g HCl/100 g solvent at 760 mm Hg, at				
	−10°C	0°C	10°C	20°C	30°C
methyl alcohol	120.26	105.33		88.68	75.43
ethyl alcohol		83.15	74.52	69.49	61.55
ethyl ether	60.02	55.28	43.57	33.15	24.17

VII. Analytical Methods

Aksel'rod developed a rapid and accurate method for the determination of free hydrochloric acid in the presence of organic acids and chlorides. He found that the addition of tropaeolin 00 produces a violet-red color with hydrochloric acid, the intensity of which increases with increasing concentration; tartaric acid gives an orange color that becomes yellow upon the addition of water; and citric acid gives a yellow color (7).

VIII. Occurrence

A. Plants

None

B. Animals

Hydrochloric acid is found in varying concentrations in the gastric juices of animals. In man the gastric juices contain about 0.5% of the acid.

C. Synthetics

None

D. Natural Inorganic Sources

The water of some rivers has been found to contain minute amounts of hydrochloric acid. The acid is also known to be present in minute quantities in volcanic areas.

Biological Data

I. Acute Toxicity

Rabbits

Hermann et al. reported that the largest non-fatal amount of 0.25 N hydrochloric acid that could be injected into 2 kg rabbits is 0.6 g/kg or 54.2 ml/kg. The pH of the blood dropped by an average of 0.35. Death occurred suddenly, accompanied by dyspnea and spasms in the tensor muscles. Injections of sodium bicarbonate as an antitoxin were unsuccessful (164).

Man

On the basis of reported cases of death from overdose with hydrochloric acid, Poison and Tattersall suggested that the lethal dose of hydrochloric acid for man is probably of the order of an ounce or less (341).

II. Short-Term Studies

Chicks

A 27-day feeding study using groups of 10 mixed-sex, one-day-old, Indian River broiler chicks was conducted by Pritzl and Kienholz. Hydrochloric acid was added to the diet of the chicks as shown in Table 1. At concentrations of 0.21 and 0.42% of the diet, the acid produced no significant effects on the chicks. However, a concentration of 0.84% resulted in a lower body weight gain and feed efficiency (351).

Table 1.—*Effect of supplemental acids in diets[1] upon total gain, feed consumption and gain/feed ratio*

Acid added to diet	Moles of acid / kg. of feed	% in the diet	pH of feed[2]	pH of feces[2]	Average 27 day total gain(g.)	Gain / feed %	Mortality %
None	0	0	5.9	6.7	535A	59	2
HCl	.06	.21	5.4	5.7	543A[3]	57	0
HCl	.12	.42	5.2	5.7	529A	59	0
HCl	.23	.84	4.6	5.6	494AB	49	0

[1]The basal diet contained (% basis) the following: 50.55 ground corn, 32 soybean meal (44% protein), 4 menhaden fishmeal, 4 soybean oil, 3 meat and bone meal, 2 brewers' dried yeast, 1.5 dehydrated alfalfa meal, 1.5 dicalcium phosphate, 0.8 ground limestone, 0.3 NaCl, and 0.35 micro-ingredients. The micro-ingredient mix suppled the following per kg. of diet: 6200 I.U. vitamin A, 1100 I.U. vitamin D_3, 105 mg. choline chloride, 33 mg. niacin, 4.4 mg. riboflavin, 14.3 mg. d-pantothenic acid, 4.2 mg. pyridoxine, 2.2 mg. vitamin K, 11 mg. vitamin E, 1.3 mg. folacin, 0.13 mg. biotin, 0.011 mg. vitamin B_{12}, 2.2 mg. thiamine, 50 mg. Mn, 50 mg. Fe, 50 mg. Zn, 5 mg. Cu, 1.5 mg. I, 0.05 mg. Co, and 1 g. methionine.

[2]The pH values represent one observation per treatment when birds were 14 days of age.

[3]Numbers without common letter are different at 1% level of significance.

Rats

To study the effect of adding hydrochloric acid to the diet, Burns conducted a 9-week feeding study using 16 rats. The rats each received about 12 ml of acid daily (0.1 N HCl) at the beginning of the experiment. This was gradually increased until at the end of 7 weeks they were each receiving 50 ml of 0.1 N acid daily, and then for the last 2 weeks they were given 50 ml of 0.2 N acid each per day. After the dose of acid reached 25 ml of 0.1 N per day, the appetites of the animals fell off and the food supply to the controls was gradually limited to approximate the amount actually consumed by the experimental groups. After 9 weeks the treated rats died and the controls were sacrificed. Results of the test are presented in Table 2. Both bone and body growth were slightly retarded by the consumption of the acid. Analysis of the bones revealed no appreciable changes in their chemical composition. The base content of the soft tissues was increased slightly in those rats in which death was preceeded by marked loss in weight (52).

TABLE 2.

					Bone analysis								Soft tissues	
Litter	Duration of exp. days	Amount of *N* HCl administered cc.	Final body-weight (g.)	Bone weight (g.)	Ash % (A)	Water %	Fat %	Organic residue % (R)	Ca %	P %	Ca/P	A/R	Base equiv. (cc. *N* alkali) per 100 g. fresh tissues	Water %
A	70	0	118	2·461	38·9	31·3	6·7	23·1	14·5	7·2	2·03	1·69	12·5	73·9
	70	0	114	2·510	34·5	37·5	5·6	22·4	13·0	6·3	2·05	1·54	12·1	75·3
	70	0	110	2·269	39·8	31·8	5·2	23·2	15·4	7·3	2·10	1·72	13·0	72·6
	70	0	104	2·313	37·8	33·4	6·5	21·8	14·3	7·0	2·03	1·73	12·3	73·2
	Mean	...	112	2·388	37·8	33·5	6·0	22·6	14·3	6·95	2·05	1·67	12·5	73·7
A	70	100–200												
	Mean (4 rats) ...		74	1·971	36·7	34·8	4·0	24·5	14·0	6·90	2·02	1·50	13·8	74·0
B	60	0												
	Mean (4 rats) ...		105	1·944	37·6	34·4	4·9	23·0	14·7	7·0	2·10	1·64	12·4	73·6
B	60	100–200												
	Mean (4 rats) ...		83	1·745	36·9	34·8	4·1	24·2	14·4	7·0	2·05	1·52	12·9	74·1

The variations in the figures for the rats in the control group, litter A, indicate the order of the variations found in the different groups.

In a study by Beznak et al., hydrochloric acid was administered to 180 g male albino rats intraperitoneally in doses equivalent to 0.75 or 1.60 times the normal H ion content of the serum (the doses were 3 or 6 mg). A total of six doses were administered over a period of 4 days. At the end of the test period the rats were sacrificed and the adrenals removed and weighed, whereupon it was observed that the adrenals exhibited considerable hypertrophy. The authors suggested that this phenomenon was a result of the elevated serum hydrogen ion concentration, since maintainence of rats on other regimens providing elevated serum hydrogen ion levels also resulted in adrenal hypertrophy (38).

Rabbits

The addition of hydrochloric acid to the diet of rabbits was carried out by Burns as outlined in Table 3. The animals receiving acid showed either retarded growth or actual weight loss, and suffered from

severe malnutrition. Compared with litter-mates on the same diet without acid, the treated rabbits showed a reduction in the base content of the muscles, but not of the viscera. Analysis of the bones revealed a marked reduction in the fat content, but no reduction in the percentage of ash (52).

Table 3

Litter	Duration of exp. days	Amount of N HCl administered cc.	Weight (kg.) Initial	Weight (kg.) Final	Bone wt. (g.)	Bone analysis: Ash % (A)	Bone analysis: Water %	Bone analysis: Fat %	Bone analysis: Organic residue % (R)	Bone analysis: Ca %	Bone analysis: P %	Bone analysis: Ca/P	Soft tissues: Base equiv. (cc. N alkali) per 100 g. muscle: Fresh	100 g. muscle: Dry	100 g. organs: Fresh	100 g. organs: Dry	Water %: Muscle	Water %: Organs
1	17	0	0·8	0·8	6·714	26·7	39·2	13·8	20·2	9·6	—	—	13·0	51·4	12·5	46·0	74·8	72·8
	17	45	0·9	0·8	7·151	25·7	50·1	5·0	19·2	9·3	—	—	11·0	43·8	10·4	43·4	74·8	76·1
2	28	0	0·5	0·45	4·400	21·0	58·1	1·4	19·4	7·7	4·2	1·85	12·9	61·1	12·4	51·2	78·9	75·8
	28	120–150	0·6	0·45	5·108	20·6	59·9	0·7	18·8	7·5	4·1	1·85	11·3	48·8	13·5	60·3	77·0	77·6
3	28	120–150	0·9	0·9	6·668	28·6	48·4	—	23·0*	10·5	5·2	2·00	14·2	62·0	11·1	42·9	77·1	74·1
4	32–35	0	0·25	0·65	4·131	19·0	47·8	—	33·2*	6·5	3·6	1·82	14·7	54·4	14·0	50·0	73·0	72·0
	32–35	0	0·20	0·55	3·515	19·0	52·3	—	28·7*	6·5	3·6	1·82	14·5	64·2	12·5	53·2	77·4	76·5
	32–35	150–200	0·20	0·40	2·994	16·8	67·9	—	15·3*	5·9	3·3	1·80	12·9	64·4	13·1	59·5	80·0	78·0
	32–35	150–200	0·25	0·50	3·546	15·7	66·8	—	17·5*	5·4	3·0	1·82	11·7	54·4	11·0	54·4	78·5	79·8
	32–35	150–200	0·30	0·60	3·881	22·0	50·4	—	27·6*	7·7	4·0	1·92	8·6	39·6	9·8	35·5	78·3	72·4

* Organic residue + fat.

III. Long-Term Studies

None

IV. Special Studies

Carcinogenicity

The injection of 0.5% hydrochloric acid subcutaneously into 8 mice was carried out by Suntzeff et al. as outlined in Table 4. The injections were given 6 times a week. While it was observed that spindle-cell sarcomas developed at or near the site of injection in 4 of the 8 mice, it was not determined whether it was the acid itself which induced the tumor or the 1.02% solution of acid potassium phthalate which was used to buffer the hydrochloric acid. Furthermore, it was pointed out that impurities present in the phthalate solution may have been the real cause of the tumor formation (439).

TABLE 4

(1) DISTILLED WATER, 0.5 c.c.: 7 mice, no sarcomas

1	male, strain	A,	18.5	mos. old,	injected for	15.5	mos.		
2	" "	D,	17.5	"	"	"	"	15.5	"
1	" "	D,	8	"	"	"	"	6	"
1	" "	C57,	16	"	"	"	"	14	"
1	" "	C57,	14	"	"	"	"	11	"
1	" "	AKA,	17.5	"	"	"	"	16	"

(2) HYDROCHLORIC ACID, 0.25 c.c., buffered to pH5 with 1.02 per cent acid potassium phthalate (KHC HO): 8 mice, 4 sarcomas at injection site

1	male, strain	A,	14	mos. old,	injected for	10.5	mos.			Sarcoma
1	" "	A,	16.5	"	"	"	"	13	"	Sarcoma
1	" "	D,	17	"	"	"	"	15	"	Sarcoma
1	" "	D,	18	"	"	"	"	16	"	No sarcoma
1	" "	C57,	15	"	"	"	"	12.5	"	Sarcoma
1	" "	C57,	18.5	"	"	"	"	16	"	No sarcoma
1	" "	AKA,	13.5	"	"	"	"	11.5	"	No sarcoma
1	" "	AKA,	17.5	"	"	"	"	15.5	"	No sarcoma

The details of the administration of hydrochloric acid, with or without 1, 2, 5, 6-dibenzanthracene, to mice in a test for carcinogenicity conducted by Dyer et al. are presented in Table 5. Upon autopsy some of the animals exhibited hemorrhagic stomachs, presumably as a result of the irritating effects of the acid. In no case did the treatment produce cancer or precancerous lesions in the gastric mucosa of the animals. One female mouse which had received 92 doses of hydrochloric acid and 11 doses of dibenzanthracene over a period of 8.5 months showed an inflammatory reaction about the base of the glands, and some hyperplastic cells (99).

TABLE 5. —*Summary of treatment of mice*[1]

Agent	Concentration[2]	Number of injections per mouse[3]		Number of mice		Age of Mice at—				Final weights of mice		Appearance of stomachs at necropsy			
						First treatment		Necropsy				Gross		Microscopic	
		Range	Average	Died	Killed	Range	Average	Range	Average	Range	Average	Normal	Hemorrhagic	Normal	Pathologic[4]
						Months	*Months*	*Months*	*Months*	*Grams*	*Grams*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Water, approximately 60° C.		15-141	57	34	28	1-11	4	3-22	11	11-41	23	92	8	93	0
Control emulsion		4-23	13			1-13	6								
Water, approximately 60° C., and.		11-120	67	14	18	1-6	3	3-17	11	10-36	24	94	6	72	0
Dibenzanthracene emulsion.	2 mg. per cc	3-16	11			1-8	5								
Water, room temperature		16-131	62	20	14	1-8	4	7-18	12	17-34	26	93	7	93	7
Control emulsion		3-23	13			1-10	5								
Water, room temperature, and.		27-138	75	15	17	1-6	2	5-18	11	15-46	29	97	3	82	12
Dibenzanthracene emulsion.	2 mg. per cc	2-17	13			1-6	3								
Hydrochloric acid	1.0 to 0.25N	10-92	41	35	23	1-7	3	2-18	9	10-45	24	88	12	65	4
Control emulsion		3-21	12			1-10	4								
Hydrochloric acid, and	1.0 to 0.25N	19-99	54	18	22	1-7	3	4-17	11	13-52	25	93	7	86	0
Dibenzanthracene emulsion.	2 mg. per cc	2-24	12			1-6	3								

[1] Mecholyl chloride was administered subcutaneously; atropine sulfate, subcutaneously and in drinking water; lactic acid in drinking water; and all other agents by esophageal intubation.
[2] The volumes administered were 0.01 cc. of agent per gram of body weight for all esophageal intubations and subcutaneous injections, and ad libitum when agent was administered in the drinking water.
[3] When agent was administered in drinking water the values are for days of treatment per mouse.
[4] Miscellaneous pathologic changes other than typical lesions in mice of strain 1.

Teratogenicity

Experiments by Landauer in which 0.05 ml of dilute (pH about 1) or concentrated hydrochloric acid was injected into the yolk of White Leghorn eggs prior to incubation had the following results. After injection of dilute hydrochloric acid, 3.5 ± 0.6% of all embryos surviving the 17th day were rumpless, while among the controls the incidence of rumplessness was 1.9 ± 0.5%. When concentrated hydrochloric acid was injected the incidence of rumplessness was 3.8 ± 1.1%, compared to 0.8 ± 0.5% among the untreated controls. No significant increase in the frequency of malformations other than rumplessness was observed. The authors concluded that while the concentrated hydrochloric acid probably produced a significant rise in the incidence of rumplessness, the dilute hydrochloric acid did not (241).

On the 13th day of gestation, Dostal injected 17 mouse fetuses intraamniotically with 2 microliters 0.1 M hydrochloric acid; 41 fetuses serving as controls received physiological saline. The results were read on the 16th day of gestation and it was found that the hydrochloric acid produced no teratogenic effects. Fetal mortality, however, was slightly (5.8%) increased by the acid injections (91).

Gastric Pain

The role of hydrochloric acid in the causation of gastric pain was studied by Hardy through tests performed on 155 patients in whom organic disease of the stomach or duodenum was demonstrable and in 73 patients in whom no local lesion could be discovered. The patients were given a dose of 200 ml of 0.5% hydrochloric acid by stomach tube. If no pain had been produced at the end of half an hour the stomach was emptied and a further 200 ml of acid introduced, and this was repeated a third time if necessary. It was found that hydrochloric acid will reproduce typical gastric pain in certain susceptible individuals suffering from painful disturbances of gastric function and that the pain occurs independently of any local disease in the stomach or duodenum. The role of hydrochloric acid in the causation of pain was discussed by the author, who concluded that the acid plays no essential part in ordinary pain production. According to Hardy, other workers had also observed that ordinary gastric pain is not associated with gastric acidity (154).

Ulcers

The effect of hydrochloric acid on the production of gastric ulcer was studied by Matzner and Windwer through the execution of the experiments outlined in Tables 6 and 7. They found that while a solution containing both pepsin and hydrochloric acid was quite effective in the production of ulcer-like gastric lesions in the albino rat, a solution of 0.3% hydrochloric acid alone was relatively ineffective in this respect, especially when administered to non-fasting animals (274).

TABLE 6

Group 1. Fasting group

Sub-Groups	No. of Rats	B. S. D. = Bills Stock Diet	Mean initial body weight gm.	Mean final body weight gm.	Duration of experiment days	Autopsy findings	No. of lesions	No. of rats without lesions	Percentage of rats with lesions %
a	30	B. S. D. + .3%. HCl + 20% Pepsin. Fasting periods 48 hrs.	190	183	12 to 21	Multiple lesions in 29 rats.	1 to 8	1	96
b	18	B. S. D. + .3% HCl. Fasting 48 hrs.	275	202	21	2 rats—3 lesions, 1 rat—1 lesion.		15	16
c	10	B. S. D. + .3% HCl + 20% inactivated Pepsin. Fasting 48 hrs.			12 to 21	Multiple lesions in 2 rats.		8	20
d	15	B. S. D. + water. Fasting periods of 48 hrs.			28			15	0

TABLE 7

Group 2. Non-fasting group

Sub-Groups	No. of Rats	B. S. D. = Bills Stock Diet	Mean initial body weight gm.	Mean final body weight gm.	Duration of experiment days	Autopsy findings	No. of lesions	No. of rats without lesions	Percentage of rats with lesions %
a	10	B. S. D. + .3% HCl. 20% Pepsin. No fasting.	165	152	13 to 21	1 rat—5 lesions. 1 rat—1 lesion.		8	20
b	10	B. S. D. + .3% HCl. No fasting.	261	237	21	Negative.	0	10	0
c	8	B. S. D. + 20% Pepsin (dry). No fasting.			28	Negative.		8	0
d	60	Additional controls on various stock diets.			21	Negative.	0	0	0

Inhalation

In an inhalation study by Higgins et al., groups of 10 rats (Wistar, 250-275 g) and 15 mice (ICR, 30-35 g) were exposed for 5 minutes to hydrogen chloride in the concentrations shown in Tables 8 and 9. Analysis of the results gave an LC_{50} of 40,989 ppm for the rats and 13,745 ppm for the mice (168).

TABLE 8 *Seven-day Mortality Response of Rats Exposed 5 min to HCl Vapors Singly and in Combination with CO (25% Carboxyhemoglobin)*

HCl concentration (ppm)	*Deaths (%)*	
	HCl	*HCl + CO*
30,000	0	
32,000	10	
33,980		10
39,850	60	
42,460		80
45,200	70	
49,580		100
56,046		90
57,290	90	
59,280		100
LC_{50}	40,989 ppm	39,010 ppm
95% confidence limits	34,803 to 48,272 ppm	35,049 to 43,419 ppm

TABLE 9 *Seven-day Mortality Response of Mice Exposed 5 min to HCl Vapors Singly and in Combination with CO (25% Carboxyhemoglobin)*

HCl concentration (*ppm*)	*Deaths (%)*	
	HCl	*HCl + CO*
3,200	7	
4,920		10
5,060	7	
6,145	13	
6,410	0	
7,525	40	
8,065	13	
9,276	33	
12,805		70
13,655	40	
21,010		80
26,485	87	
27,386		90
30,000	87	
LC_{50}	13,745 ppm	10,663 ppm
95% confidence limits	10,333 to 18,283 ppm	6,921 to 16,428 ppm

The effect of the inhalation of hydrogen chloride was studied by Machle et al. using rabbits, guinea pigs, and monkeys. The animals were exposed to the gas in concentrations ranging from 0.05 to 20.5 mg/l for periods ranging from 5 to 7200 minutes. A concentration of 6.5 mg/l resulted in 100% fatality in 30 minutes and 1 mg/l gave the same result if the exposure was of long duration (2-6 hours). The effect of exposure on the growth of the animals was not remarkable; a sharp, but temporary (2-4 week), loss of weight was evident in every instance. Histopathological examination disclosed that the primary toxic effect of the gas was its corrosive action on the respiratory tract. In addition to the respiratory damage, the liver, kidneys and heart of animals exposed to high concentrations showed degenerative changes, which were described in detail by the authors (refer to attached article) (264).

Fatal Overdose

Because of the immediate pain when taken into the mouth, the consumption of strong solutions of hydrochloric acid occurs only rarely. In the period between 1828 and 1951, 140 cases of the ingestion of corrosive acid were reported (207). These cases involved infants, inebriated individuals, or those attempting suicide. As it would be impracticle to incorporate all of these reports into the current monograph, their observations are summarized and only a few representative studies are included.

Upon ingestion of concentrated hydrochloric acid, the immediate clinical manifestations are burning of the mouth and lips, frothing, and spitting, followed shortly by gagging and vomiting. The vomitus is likely to contain shreds of mucosa and to be bloody. The acute reactions persist for several days, then gradually subside (207, 462).

The acid generally passes rapidly through the esophagus causing

little damage. However, second-degree burns are occasionally produced in which necrosis of the mucous membranes and muscular layer takes place. The necrotic tissue sloughs and granulations form. Unless the formation of scar tissue can be prevented or controlled, a stricture of the esophagus will develop. It has been estimated that 6 to 20% of patients who developed pyloric stenosis due to acid ingestion have an associated esophageal stricture (462, 243, 325).

Upon entering the stomach, the acid rapidly passes along the lesser curvature of the stomach to the distal end whereupon it induced tetanic contractions of the pylorus and is trapped and maintained in long contact with the distal gastric mucosa. It is at this point that the acid causes its greatest damage. Less concentrated solutions cause extensive necrosis before they are neutralized. Highly concentrated acid, however, may cause perforation and early death. If the patient survives the immediate effects, vomiting ceases in a few days, oral lesions disappear and within a week or ten days the patient feels well and apparently has recovered (462, 137, 21).

However, 3-4 weeks after ingestion, vomiting recurs and becomes persistant. The patient suffers epigastric pain which is usually a mild sense of fullness and pressure after meals. The free hydrochloric acid is generally low or absent. There is loss of weight. The roentgenogram reveals almost complete pyloric obstruction. Thus, the end result of ingestion of acid is permanent pyloric stenosis that may lead to death unless corrected surgically (207, 462, 29, 21, 347).

The severity of damage produced by acid ingestion varies with the quantity and concentration of acid consumed as well as the state of the stomach contents. It has been suggested that the relatively more hazardous solutions are those which are more dilute and are tolerated by the stomach rather than expelled. The exposure time of the mucous membranes to such solutions is prolonged, resulting in more severe injury (29).

There is some disagreement as to the effect of the stomach content on the action of the acid. Several workers have stated that when the acid is swallowed on a full stomach, food prevents contact with all but the lesser curvature and pars pylorus, and when taken when the stomach is empty, the fluid comes in more general contact with the gastric mucosa (21, 207, 347). Other workers have attested to the opposite: that the damage is more diffuse when the stomach is full (137, 325, 431).

An unusual case of fatal acid ingestion was reported by Velten. The case involved a 48-year-old women who attempted suicide by drinking 200 g household hydrochloric acid. Two hours after the attempt the woman was admitted to a clinic in a high degree of shock, with imperceptible pulse, cold extremities, and scale in the mouth and oral cavity. The stomach was pumped out and subsequently rinsed with milk. Initially the woman responded with good recovery and clear consciousness, but 10 hours after admission developed sudden nausea and died within a few minutes. Upon autopsy was found extensive acid scaling in the lowest portion of the esophagus, in the stomach, and in the duodenum; slight scaling of

the upper portion of the esophagus. Microscopic examination of the brain revealed: occasionally marked bloating of the cells, also of the nuclei; eccentric displacement of the nuclei; swelling of the dendrites; sometimes a fine-grained decomposition of the cells with shrinkage of the cell bodies; partially pronounced tigrolysis; shadow cell formation; pseudoneuronophagia and also genuine neuronophagia; noticeable homogenization of the cytoplasm with complete disappearance of the structure, particularly in the dome area. Coagulation processes were involved; the coagulated cell protoplasm appeared to strain dark green in Nissl's preparation. Upon microscopic examination of the spinal column it was found that the large motor ganglia cells also showed swelling of the cell bodies, eccentric displacement of the cell nuclei, and fine grained cell decomposition. A peculiar homogenization of the blood, as in the case of stasis, was observed in the vessels of the spinal column. The author speculated that these effects were the result of "an absorptive protein autointoxication" caused by the absorption of protein decomposition products produced in the acid scaling of the mucosa (471).

Biochemical Aspects

I. Breakdown

No Information Available

II. Absorption-Distribution

The absorption of hydrochloric acid by the human stomach was studied by Shay et al. through gastric intubation of 200 ml of a solution of approximately 0.5 or 1% hydrochloric acid into fasted patients. Thirty minutes after the gastric instillation the stomach was emptied and the contents analyzed. It was found that the hydrochloric acid was not absorbed by the stomach under these conditions (419).

III. Metabolism and Excretion

The human stomach normally contains hydrochloric acid to the extent that the pH is maintained at 1.5-2.5. When it passes into the duodenum, the acid from the stomach is neutralized by alkaline secretions from the pancreas and liver (423, 209).

It is reasonable to assume, since the addition of hydrochloric acid to the stomach proportionally inhibits secretion of the acid (329, 419), that when the acid is ingested in small amounts it is handled by the body through the ordinary mechanism. When large quantities are consumed, excess acid may be absorbed, resulting in acidosis. This phenomenon and the mechanisms whereby it is corrected are discussed in the following section.

IV. Effects on Enzymes and Other Biochemical Parameters

When hydrochloric acid is ingested, an excess of acid or of acid products may be absorbed, resulting in metabolic acidosis. Studies in acid-base equilibrium have shown that when this occurs neutralization is immediately effected by buffer salts and mainly by the alkali bicarbonate of the plasma. As a result, the normal bicarbonate-carbonic acid ratio of 27/1.35 decreases and the pH may fall to 7.0 or lower (138, 452).

The decreased pH stimulates the respiratory center, and an increased depth and rate of respiration occur in an attempt to lower the carbonic acid concentration and restore the pH to its normal value. In addition the following renal compensatory mechanisms may develop: (a) an increased secretion and excretion of hydrogen ions; (b) stimulation of ammonium, and excretion of ammonium ions; (c) retention of bicarbonate, and excretion of chloride ions instead; (d) a slight decrease in sodium excretion because of the conservation and retention of bicarbonate, possibly resulting in a slight rise in the serum sodium concentration; (e) conversion of monohydrogen phosphate into dihydrogen phosphate, and excretion of the latter (138, 452).

As a result of these compensatory changes, namely, the pulmonary elimination of carbon dioxide and the renal retention of bicarbonate, the bicarbonate-carbonic acid ratio and the pH rise toward normal (138, 452).

The compensatory hyperventilation which occurs as a result of the metabolic acidosis may persist even after the patient is no longer acidotic. The reason for this is not known. However, this observation is important because the patient who is recovering from metabolic acidosis can develop a serious respiratory alkalosis due to the hyperventilation which continues (138).

A mild metabolic acidosis may be asymptomatic. Symptoms usually appear when the CO_2 content falls to 18.2 mEq/l (40 vol %), or lower. The patient may complain of weakness or general malaise, or dull headache. In addition, nausea, vomiting, and abdominal pain may be present. Characteristic very deep respirations (Kussmaul breathing, air hunger) are often present. The respiratory rate is also usually increased, but may be slow. The increased depth of respiration is more characteristic of acidosis than the increased respiratory rate. These signs are usually present when the CO_2 content falls below 9.1 mEq/l (below 20 vol %), and the pH is 7.2 or lower. When the pH falls to 7.0, respiratory depression may occur. Examination of the electrocardiogram often reveals signs of hypopotassemia. Severe acidosis can also cause RS-T deviations of the type usually found in myocardial injury. An excess elimination of water and neutral salts by the kidneys may lead to dehydration and possibly to some loss in vital activity of the tissues. A leukocytosis may be present. The urine of acidotic subjects has a high ammonia content and is very acid (pH 4.6 to 6.2) (138, 452).

Through an extensive series of experiments, Fazekas found that the development of acidosis through the continued administration of hydrochloric acid can cause the following results in rabbits: hypertrophy and increased function of the adrenal cortex; enlargement of the ovaries and uterus; follicle maturity and follicle hemorrhage; corpora lutea formation; cyclic changes of the mucous membrane of the uterine horns; enlargement and increased function of the parathyroids; enlargement of the pituitary gland and multiplication of the basophilic cells of the anterior lobe of the pituitary. The author contends that the administration of acid primarily produces the observed effect on the pituitary gland and that it in turn produces the other effects through increased hormone production (111, 113, 114).

The influence of acid-feeding on the utilization of mineral elements was examined by Telfer through an experiment in which a child of 6 was fed, for a period of 6 days, exclusively a measured amount of milk to which sufficient hydrochloric acid had been added to give a concentration of 0.05 N. The analysis of the excreta was compared with that of excreta over similar periods, before and after the experimental period, when no hydrochloric acid was added to the milk. A higher degree of absorption of Ca, Mg and P due to the acid was indicated by an increase in the renal output of CaO, MgO and P_2O_5. The quantities of these minerals which were retained were slightly diminished. The author inferred

from these results that hydrochloric acid adversely affects the process of calcification, presumably through the production of acidosis. In a discussion of his results, Telfer mentioned that earlier investigations had also shown that the administration of hydrochloric acid is followed by an increased urinary excretion of Ca and P. The author postulated that the increased absorption of minerals was effected by increasing the acidity of the duodenum and thereby allowing the dietary minerals which are insoluble, and consequently not absorbed, in the normally alkaline duodenum to be maintained in a soluble form for a longer period and over a larger area of the duodenum (452).

Instillation of hydrochloric acid into the stomach of fasting anesthetized dogs was reported by Code et al. to inhibit gastric absorption of sodium but to have no effect on the absorption of water and potassium from the stomach (71).

Upon oral administration of 3.5 g hydrochloric acid daily for 3 days to human subjects, Stehle and McCarty observed increased urinary excretion of potassium, sodium, ammonia, phosphoric acid and hydrogen ions (430).

Tests by Benyo et al. showed that intraduodenal injection of hydrochloric acid (0.35%; 2.5-3.0 ml/kg) into anesthetized dogs resulted in significantly increased hepatic circulation. This effect lasted 5-16 minutes, during which time there was a small but definite lowering of blood pressure (34).

Deloyers and Suprez reported that hydrochloric acid exhibits a strong hypoglycemic effect when administered to man intraduodenally, the effect reaching its maximum in one hour after administration (84). Boattini, however, found that while hydrochloric acid exhibits a strong hypoglycemic effect on patients with diabetes mellitus, only a very slight effect is manifested in normal subjects (40).

It has been well established that the introduction of hydrochloric acid into the stomach proportionately depresses its own secretion in the stomach (329, 419). Inhibition of gastric secretion is also observed when hydrochloric acid is intubated into the duodenum (11, 203).

Johnson studied the effect of hydrochloric acid on pepsin secretion using 4 dogs with vagally denervated pouches of the oxyntic gland area. Irrigation of the pouches with 200 mN hydrochloric acid caused a 40% rise in pepsin output, 250 mN caused a 75% rise, and 300 mN caused a 300% rise over control levels obtained during irrigation with 100 mN hydrochloric acid. The author suggested the following explanation for this phenomenon. Normally the gastric mucosa is relatively impermeable to hydrogen ions. However, high concentrations of acid damage the mucosal barrier and allow excessive back diffusion of H ions. The results of the tests indicate that pepsin is secreted in response to the back diffusion of H ions, the amount of pepsin secreted being directly related to the amount of H ions lost from the pouch. When sufficient acid is used to break the mucosal barrier, large increases in pepsin output occur. The threshold concentration of hydrochloric acid which damages the barrier was established as somewhere between 250 and 300 mN (201).

A number of workers have reported that the addition of hydrochloric acid to test meals inhibits gastric motility and slows gastric emptying, this effect being mediated through duodenal receptors which are stimulated by the acid as it leaves the stomach (179, 329). The threshold for this effect was reported to be about 5-10 mN, the effect then increasing proportionally with increasing concentrations of acid (179).

Other workers, however, have stated that the acidity of the contents of the stomach and duodenum has no effect on the emptying time of the stomach (47, 277, 279).

With great enthusiasm, MacGilvra reported that Pearl L. Moorman had found that small amounts of intravenously administered hydrochloric acid were effective in accelerating the recovery of anesthetized (ether) rabbits and that a patient moribund as a result of an unusual reaction to avertin was rapidly revived by intravenous hydrochloric acid. Dogs, cats, and monkeys tested by MacGilvra lent further support to the "palinesthesic" effect of the acid (104, 263).

In response to the report by MacGilvra (263) that intravenously administered hydrochloric acid accelerates termination of narcosis, Shambaugh and Boggs conducted a series of studies to test this phenomenon. In a preliminary study using dogs anesthetized with nembutal intraperitoneally, avertin by rectum, or ether by inhalation, they found that doses of 3 to 65 ml of 0.1 N hydrochloric acid had no effect on the duration of narcosis (416). The results of a more thorough study by Shambaugh and Boggs in which guinea pigs, rabbits, dogs, rhesus monkeys and baboons were anesthetized with ether, nembutal, or avertin indicate that injections of up to 350 ml of 0.1 N hydrochloric acid demonstrated no effect on the rate of recovery (415).

Upon conducting a similar set of tests, Kinard and van de Erve reported that i.v. injections of 0.1 N hydrochloric acid (1-10 ml) did not hasten the recovery of dogs and rabbits from nembutal, ether or paraldehyde anesthesia (216).

In experiments performed on cats and dogs, Chandler and Chopra observed that hydrochloric acid increases the toxicity of CCl_4 when both are administered orally (67).

VI. Consumer Exposure

The following tables were compiled in 1972 from data submitted by user firms. Food consumption values for each food category were derived from the Market Research Corporation of America (MRCA) data on frequency of eating and from the USDA data on mean portion size of foods in each food category. The food consumption values thus derived were coupled with the usage level data obtained in the surveys to calculate the daily intake of each substance.

TABLE 2 -- USAGE LEVELS REPORTED FOR NAS APPENDIX A SUBSTANCES (GROUP I) USED IN REGULAR FOODS(R)

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO.	NAME	# FIRMS REPORTING	*** USUAL USE *** WTD MEAN, %	*** MAXIMUM USE *** WTD MEAN, %
HYDROCHLORIC ACID NAS 0097	01	BAKED GOODS(R)	*	.00200	.00370
	06	CHEESE(R)	*	.00200	.00200
	08	PROCSD FRUT(R)	*	.00490	.00670
	19	SWEET SAUCE(R)	7	.27341	.27375
	20	GELATIN PUD(R)	*	********	********
	23	BEV TYPE I(R)	*	.00250	.00370
HYDROCHLORIC ACID NAS 0097	(CONT.) 26	RECONST VEG(R)	*	********	********

TABLE 11, PART A -- ANNUAL POUNDAGE DATA FOR NAS APPENDIX A SUBSTANCES (GROUPS I & II)

SUBSTANCE NAME (SURVEY NO.)	# REPORTS TO NAS 1969/1970	POUNDAGE REPORTED TO NAS (MATCHING REPORTS FOR BOTH YEARS) 1969	1970	TOTAL 1970 POUNDAGE REPORTED TO NAS	# REPORTS TO FEMA	POUNDAGE REPORTED TO FEMA-- 1970 ONLY	TOTAL 1970 POUNDAGE NAS + FEMA
HYDROCHLORIC ACID NAS 0097	16/ 17	4,142,000	37,646,810	37,946,810			37,946,810

TABLE 13, PART A -- POSSIBLE DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUPS I & II), PER FOOD CATEGORY AND TOTAL DIETARY, BASED ON FOOD CONSUMPTION BY TOTAL SAMPLE - - - SEE EXPLANATORY NOTES IN EXHIBITS SECTION

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO. NAME	# OF FIRMS	(AGE)	POSSIBLE DAILY INTAKE, MG. AVERAGE	HIGH A	HIGH B
HYDROCHLORIC ACID NAS 0097	01 BAKED GOODS(R)	*	0-5 MO.	.068000	.090000	.125800
			6-11 MO.	.508000	1.036000	.939000
			12-23 MO.	1.090000	1.796000	2.016500
			2-65+ YR.	2.744000	4.076000	5.076400
HYDROCHLORIC ACID NAS 0097	06 CHEESE(R)	*	0-5 MO.	**********	.002000	**********
			6-11 MO.	.054000	.194000	.054000
			12-23 MO.	.156000	.444000	.156000
			2-65+ YR.	.188000	.472000	.188000
HYDROCHLORIC ACID NAS 0097	08 PROCSD FRUT(R)	*	0-5 MO.	.230300	.617400	.314900
			6-11 MO.	2.538200	6.321000	3.470600
			12-23 MO.	4.929400	9.785300	6.740200
			2-65+ YR.	5.796700	12.279400	7.926100
HYDROCHLORIC ACID NAS 0097	19 SWEET SAUCE(R)	7	0-5 MO.	.820230	1.003640	.821250
			6-11 MO.	2.460690	8.475710	2.463750
			12-23 MO.	7.108660	20.779160	7.117500
			2-65+ YR.	18.591880	48.940390	18.615000
HYDROCHLORIC ACID NAS 0097	20 GELATIN PUD(R)	*	0-5 MO.	**********	**********	**********
			6-11 MO.	**********	**********	**********
			12-23 MO.	**********	**********	**********
			2-65+ YR.	**********	**********	**********
HYDROCHLORIC ACID NAS 0097	23 BEV TYPE I(R)	*	0-5 MO.	.060000	.090000	.088800
			6-11 MO.	.567500	1.942500	.839900
			12-23 MO.	1.355000	4.062500	2.005400
			2-65+ YR.	2.600000	6.942500	3.048000
HYDROCHLORIC ACID NAS 0097	26 RECONST VEG(R)	*	0-5 MO.	**********	**********	**********
			6-11 MO.	**********	**********	**********
			12-23 MO.	**********	**********	**********
			2-65+ YR.	**********	**********	**********
HYDROCHLORIC ACID NAS 0097	83 FORMULAS(B)	*	0-5 MO.	**********	**********	**********
			6-11 MO.	**********	**********	**********
			12-23 MO.	**********	**********	**********
HYDROCHLORIC ACID NAS 0097	ALL CATEGORIES	12	0-5 MO.	1.178530	1.893040	2.350750
	**********		6-11 MO.	6.126390	17.969210	7.788090
	**********		12-23 MO.	14.639060	36.666960	18.035600
	**********		2-65+ YR.	29.920580	72.710290	35.653500

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Aksel'rod-'62
Bib. no. 7

Vopr. Pitaniya 21(6):84-85. 1962.

DETERMINATION OF FREE HYDROCHLORIC ACID IN THE PRESENCE OF ORGANIC ACIDS AND CHLORIDES

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We did work on determining the content of hydrochloric acid in an extract of coloring from grape residues, which were used in the liquor, nonalcoholic, and even the confectionery industries. In this connection, the necessity arose for working out a sufficiently sensitive method of quantitatively determining hydrochloric acid in a large excess of tartaric or citric acid which would not change the presence of chlorides.

It is possible to back-titrate the hydrochloric acid, not affecting the tartaric and citric acid, thanks to a sharp difference in their strengths. However, an immediate titration of hydrochloric acid turned out to be unsuitable due to the washing the end of the titration.

We setablished that tropeolin OO colored the hydrochloric acid a violet-red color, the intensity of which increases with an increasing hydrochloric acid concentration. Tartaric acid colors the tropeolin OO solution an orangey color which, when diluted with distilled water, changes to yellow, approaching with dilution the color which tropeolin OO gives with distilled water. Citric acid with tropeolin OO has a yellow color similar to that in distilled water. On the basis of this, a colorimetric determination may be developed for hydrochloric acid in mixtures with tartaric or citric acid.

Detrmination of Hydrochloric Acid in a Tartaric or Citric Acid Base

Given 0.2N HCl (in ml)	Determined in a Base of: 5 ml 0.2N tartaric acid	5 ml 0.2N citric acid	Absolute Error for tartaric acid	for citric acid
0,15	0,25	—	+0,1	—
0,2	0,1	—	—0,1	—
0,2	0,15	0,2	—0,05	—
0,25	0,25	—	—	—
0,3	0,3	—	—	—
0,3	0,35	—	+0,15	—
0,4	0,35	0,4	—0,05	—
0,4	—	0,4	—	—
0,5	0,45	—	—0,05	—
0,6	0,7	0,65	—0,1	—0,05
0,6	0,55	0,6	—0,05	—
0,6	0,5	—	—0,1	—
0,6	0,35	—	—0,25	—
0,65	0,55	—	—0,1	—
0,7	—	0,7	—	—
0,7	0,7	0,7	—	—
0,8	0,85	0,7	+0,05	—0,1
0,8	0,8	0,8	—	—
0,8	0,8	—	—	—
0,9	0,9	0,95	—	+0,05
1	0,95	1	—0,05	—
1,2	1,5	—	+0,3	—
2	2	—	—	—

However, with a change in hydrochloric acid content, not only the intensity of the red color changes in the tropeolin OO solution, but the nature of the hue - from violet to orangey-yellow. In this connection, we chose the color-scale method and worked out the following analytic procedure.

In ten test tubes of the same diameter, 5 ml of 0.2N tartaric or citric acid was poured, one drop of tropeolin OO was added, and 0.1, 0.2, etc. ml of 0.2N hydrochloric acid was poured in. The volume contained in the test tubes was 10 ml. For tartaric acid, two scales were prepared: one for 0.1 to 1 ml, and one for 1 to 2 ml 0.2N HCl. For citric acid, a scale for 0.1 to 1 ml of 0.2N HCl was prepared in each test tube.

In a test tube of the same diameter, unknowns were taken for the problems studied to produce a quantitative analysis of the 0.2N HCl within the scale limits. For each problem, 5 ml of 0.2N tartaric or citric acid was poured in corresponding to one drop of tropeolin OO and the volume contained in the test tube was brought up to 10 ml with the addition of distilled water. The color of these solutions was compared with the prepared color scale, using a three-cell colorimetric comparator. The data obtained and the absolute error are presented in the table.

For the reproduction characteristics of the method, we made 10 determinations on 0.3 ml of 0.2N HCl in 5 ml of 0.2N tartaric acid. With the greatest experimental error, for 0.05 ml, we found more or less the original value of 0.004 g HCl in its over-all concentration in a 0.006N solution.

For the accuracy characteristics of the method, the mean deviations were calculated according to the equation

$$d_m = \frac{\Sigma [d]}{n},$$

the sum of the absolute errors, where n is the number of measurements, and the mean square error is

$$\sigma = \sqrt{\frac{\Sigma d^2}{n - 1}} \quad + \text{ or } - 1.25\, d_m$$

(V. N. Alekseyev[1])

The mean deviations and mean square errors calculated from these equations were, respectively, for a determination of hydrochloric acid in a tartaric acid base: d = 0.0714 and σ = + or - 0.0893, and for a citric acid base: d = 0.0167 and σ = + or - 0.0209.

From the data presented, it is evident that the method described is sufficiently accurate, rapid, and especially efficient for serial determinations of small amounts of hydrochloric acid in the presence of chlorides in a base of a whole series of organic acids weaker than tartaric acid, i.e. practically all of the organic acids occurring in food products. The scale is stable for 1½-2 hours, after which it must be restored again with the addition of one drop of tropeolin OO.

The advantage of the method is the sufficiently wide interval in the ratio of hydrochloric acid to the organic acid, from 1:5 to 1:25. A disadvantage is the difficulty in colorimetric analysis under artificial light.

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Akbel'rod-'62
Bib No. 7

Vopr. Pitaniya. 21(6):84-85. 1962.

ОПРЕДЕЛЕНИЕ СВОБОДНОЙ СОЛЯНОЙ КИСЛОТЫ В ПРИСУТСТВИИ ОРГАНИЧЕСКИХ КИСЛОТ И ХЛОРИДОВ

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Мы провели работу по определению содержания соляной кислоты в экстракте окрасителя из виноградных выжимок, который используют в ликероводочном, безалкогольном, а также кондитерском производстве. В связи с этим возникла необходимость в разработке достаточно чувствительного метода количественного определения соляной кислоты при большом избытке винной или лимонной кислоты, которому не мешало бы присутствие хлоридов.

Оттитровать соляную кислоту, не затрагивая винную и лимонную кислоту, возможно благодаря резкому различию в их силе. Однако непосредственное титрование соляной кислоты оказалось неприемлемым из-за размытости конца титрования.

Мы установили, что тропеолин 00 окрашивается соляной кислотой в фиолетово-красный цвет, интенсивность которого возрастает с увеличением концентрации соляной кислоты. Винная кислота окрашивает раствор тропеолина 00 в оранжевый цвет, который при разбавлении дистиллированной водой переходит в желтый, приближаясь с разбавлением к той окраске, которую дает тропеолин 00 с дистиллированной водой. Лимонная кислота с тропеолином 00 имеет желтую окраску подобную окраске в дистиллированной воде. На основании этого можно провести колориметрическое определение соляной кислоты в смеси с винной или лимонной кислотой.

Определение соляной кислоты на фоне винной и лимонной кислот

Взято 0,2 N соляной кислоты (в мл)	Определено на фоне		Абсолютная ошибка	
	5 мл 0,2 N винной кислоты	5 мл 0,2 N винной кислоты	для винной кислоты	для лимонной кислоты
0,15	0,25	—	+0,1	—
0,2	0,1	—	—0,1	—
0,2	0,15	0,2	—0,05	—
0,25	0,25	—	—	—
0,3	0,3	—	—	—
0,3	0,45	—	+0,15	—
0,4	0,35	0,4	—0,05	—
0,4	—	0,4	—	—
0,5	0,45	—	—0,05	—
0,6	0,7	0,65	+0,1	+0,05
0,6	0,55	0,6	—0,05	—
0,6	0,5	—	—0,1	—
0,6	0,35	—	—0,25	—
0,65	0,55	—	—0,1	—
0,7	—	0,7	—	—
0,7	0,7	0,7	—	—
0,8	0,85	0,7	+0,05	—0,1
0,8	0,8	0,8	—	—
0,8	0,8	—	—	—
0,9	0,9	0,95	—	+0,05
1	0,95	1	—0,05	—
1,2	1,5	—	+0,3	—
2	2	-	—	—

Однако с изменением содержания соляной кислоты меняется не только интенсивность красной окраски раствора тропеолина 00, но и характер оттенка — от фиолетового к оранжево-желтому. В связи с этим мы избрали метод цветной шкалы и разработали следующую методику анализа.

В 10 пробирок одинакового диаметра наливали по 5 мл 0,2 N винной или лимонной кислоты, добавляли по одной капле тропеолина 00, приливали по 0,1, 0,2 мл и т. д. 0,2 N соляной кислоты. Объем содержимого пробирок доводили до 10 мл. Для винной кислоты готовили 2 шкалы: одну от 0,1 до 1 мл, другую — от 1 до 2 мл 0,2 N соляной кислоты. Для лимонной кислоты готовили шкалу от 0,1 до 1 мл 0,2 N соляной кислоты в каждой пробирке.

В пробирке того же диаметра были взяты в качестве задач неизвестные для производящего анализ количества 0,2 N соляной кислоты в пределах шкалы. К каждой задаче приливали по 5 мл 0,2 N винной или лимонной кислоты соответственно по одной капле тропеолина 00 и объем содержимого пробирок доводили добавлением дистиллированной воды до 10 мл. Окраску этих растворов сравнивали с приготовленной цветовой шкалой, колориметрируя с помощью трехместного компаратора. Полученные данные и абсолютные ошибки сведены в таблицу.

Для характеристики воспроизводимости метода мы провели 10 определений 0,3 мл 0,2 N соляной кислоты в 5 мл 0,2 N винной кислоты. При наибольшей ошибке определения, составляющей 0,05 мл, мы находим больше или меньше истинного значения на 0,004 г соляной кислоты при ее общей концентрации в растворе 0,006 N.

Для характеристики точности метода были вычислены средние отклонения по формуле.

$$d_{ср} = \frac{\Sigma|d|}{n},$$

сумма абсолютных ошибок, n — число измерений и средняя квадратичная ошибка

$$\sigma = \sqrt{\frac{\Sigma d^2}{n-1}} \approx \pm 1,25\, d_{ср}. \qquad \text{(В. Н. Алексеев)}^1$$

Вычисленные по этим формулам средние отклонения и средние квадратичные ошибки составляют соответственно для определения соляной кислоты на фоне винной $d = 0,0714$ и $\sigma = \pm 0,0893$; на фоне лимонной — $d = 0,0167$ и $\sigma = \pm 0,0209$.

Из приведенных данных видно, что описываемый метод достаточно точен, быстр и особенно выгоден при серийных определениях небольших количеств соляной кислоты в присутствии хлоридов на фоне целого ряда органических кислот, более слабых, чем винная, т. е. практически почти всех органических кислот, встречающихся в пищевых продуктах. Шкала устойчива в течение 1½—2 часов, после чего она может быть восстановлена вторично добавлением по одной капле тропеолина 00.

Преимуществом метода является довольно широкий интервал соотношений соляной и органических кислот — от 1 : 5 до 1 : 25, недостатком его следует считать затруднительность колориметрирования при электрическом освещении.

Поступила 9.XI 196[illegible] г.

[1] В. Н. Алексеев. Количественный анализ Госхимиздат, 1959.

ОПЕЧАТКИ

В статье Л. А. Колесинской и Д. Д. Личиковой «К вопросу об определении влажности хлеба», напечатанной в № 1 за 1962 г., в выводах (4) следует читать: «Определение влажности хлеба по ОСТ ВКС 5540, занижая цифровые показатели влажности, укладывает в рамки ГОСТ хлеб с повышенной влажностью и некачественной органолептикой».

В статье Ф. С. Околова и С. Х. Николова «О водопоглощаемости и студнеобразовании мышечной ткани рыб и убойных теплокровных животных», напечатанной в № 2 за 1962 г.

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Gastroenterology 41:486-490, 1961.

INHIBITION OF POSTPRANDIAL GASTRIC SECRETION IN PAVLOV POUCHES BY INSTILLATION OF HYDROCHLORIC ACID INTO THE DUODENAL BULB

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It is well established that instillation of hydrochloric acid or gastric juice into the duodenum can inhibit gastric secretion;[1-7] however, the duodenal pH has to be depressed below 2.5 in order to elicit inhibition.[3,5,7] Since a pH as low as this has not been observed in the duodenum under physiologic conditions, the physiologic significance of the duodenal acid inhibitory mechanism has been seriously questioned.[3] Yet it does not seem unreasonable to assume that the postprandial pH in the duodenum may vary with the distance from the pylorus, values being low in the bulb and greater in distal parts. Previous determinations of pH in the duodenal contents have not permitted observation of such a gradient under physiologic conditions, for regurgitation of buffering contents from the lower into the upper duodenum has not been ruled out.

The localization and extension of the duodenal area from which inhibition is elicited by a low pH are unknown. Provided that the inhibitory area is localized to the duodenal bulb, a low postprandial pH in this juxtaventricular area might play a role in the physiologic regulation of gastric acid secretion. In the present experiments, therefore, the duodenal bulb was isolated and the inhibitory effect of the instillation of hydrochloric acid into it was investigated.

Methods

Three adult mongrel dogs weighing 15 to 25 kg. were used.

Operative procedures. At an initial operation Pavlov pouches were created; 2 to 3 weeks later a mucosal diaphragm was constructed at the pylorus. The gastrointestinal continuity was reestablished by gastrojejunostomy. At a third operation the duodenum was transected just proximal to the entrance of the common bile duct. The distal end was closed and the bulb was anastomosed end-to-end with a resected loop of terminal ileum which was brought out to the abdominal wall to form a cutaneous fistula (fig. 1A).

All operations were performed under aseptic precautions with pentobarbital sodium anesthesia (25 to 40 mg. per kg. of body weight). For the postoperative management and subsequent care of the animals, the procedure described by Uvnäs *et al.*[8] was followed. Between operations and before the initiation of the experiments the dogs were allowed a period of 2 to 3 weeks for recovery.

Experimental procedures. Each experiment was started in the forenoon after the animals had fasted for 18 to 24 hours. The basal secretion from the pouches was recorded for at least 1 hour before the secretion was stimulated by a test meal consisting of 100 gm. of minced lean meat. The secretory response during 4 hours was collected in 15-minute portions and the amounts of free and total acid were determined by titration of each portion against 0.01 N NaOH, with Töpfer's reagent and phenolphthalein as indicators.

Perfusions of the duodenal bulb were performed as follows: Through the cutaneous fistula a plastic catheter (about 3 mm. thick) was advanced into the bulb. To avoid increased pressure in the bulb during perfusions a rubber catheter (about 5 mm. thick) was also introduced through the fistula (fig. 1B). In all experiments in this

Received May 19, 1961. Accepted June 13, 1961.

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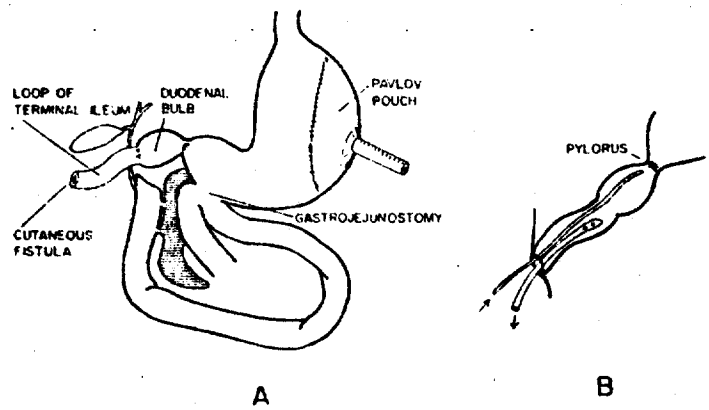

FIG. 1. *A*, operative preparation of the dogs: Pavlov pouch, mucosal wall at the pylorus, gastrojejunostomy, cutaneous fistula to the isolated duodenal bulb. *B*, mode of perfusion of the isolated duodenal bulb.

series the perfusion began simultaneously with feeding and was continued for 1 to 1½ hours. The perfusion rate was about 100 ml. per hour. During perfusions pH determinations of the effluent perfusate were made every 15 minutes. With physiologic saline the pH of the effluent perfusate varied between pH 7.0 and 7.7; with acid, between pH 1.3 and 1.6.

Three types of experiments were performed: (1) controls with no perfusion of the bulb; (2) experiments in which physiologic saline was instilled; and (3) a third run in which 0.1 N HCl was instilled into the bulb.

Results

In all, 38 experiments were performed on 3 dogs. They consistently revealed a marked inhibition of the postprandial acid secretion when the isolated duodenal bulb was perfused with 0.1 N HCl (table 1). The experiments on dog 148 show that instillation of HCl depressed the gastric secretion to about 25 per cent of the control values (fig. 2 and table 1). Instillation of 0.9 per cent NaCl solution in this dog did not produce any significant change of the HCl secretion. In dog 175 physiologic saline seems to have had some inhibitory influence, although it was slight in comparison with the action of HCl. In dog 165, on the other hand, instillation of saline increased the response somewhat above the controls. On the whole,

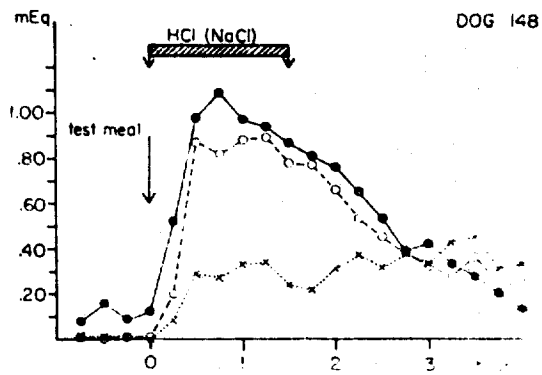

FIG. 2. Inhibition of postprandial gastric secretion from a Pavlov pouch by instillation of HCl into the duodenal bulb. ●——●, control (5 experiments); O——O, 0.9 per cent NaCl into the duodenal bulb (4 experiments); X- - - - - -X, 0.1 N HCl into the duodenal bulb (4 experiments)

perfusion of the duodenal bulb with physiologic saline did not significantly affect the postprandial secretion. In all 3 dogs, as is evident from table 1, the inhibition caused by HCl in the duodenal bulb amounted to 70 to 75 per cent. The results did not differ essentially when the secretion was expressed in milliequivalents of free HCl instead of milliequivalents of total acid.

Discussion

The results leave no doubt that a low pH in the duodenal bulb can exert a strong inhibitory influence on the postprandial acid

TABLE 4 *Continued*

Procedure and Experiment No.	Secretion (mEq. Total Acid)					
		Response to test meal				
	Control period (1 hr.)	1st hr.	2nd hr. ½ hr.	2nd hr. ½ hr.	3rd hr.	4th hr.
Dog 165						
0.9% NaCl in the duodenal bulb during the 1st and half of the 2nd hr.						
1	0.76	**4.85**	**3.43**	3.07	5.18	4.26
2	0.28	**4.26**	**2.51**	2.42	3.59	2.92
3	0.66	**4.02**	**1.45**	1.46	2.96	1.83
4	0.32	**5.39**	**3.55**	3.19	4.29	3.22
Mean	0.51	**4.63**	**2.74**	2.54	4.01	3.06
In per cent of mean for controls	—	110	112	126	97	71
0.1 N HCl in the duodenal bulb during the 1st and half of the 2nd hr.						
1	0	**1.13**	**0.59**	0.63	0.97	0.73
2	0.41	**1.80**	**2.06**	2.47	3.79	3.47
3	0.91	**0.75**	**1.03**	1.52	2.89	3.20
Mean	0.44	**1.23**	**1.23**	1.54	2.55	2.43
In per cent of mean for controls	—	29	50	76	62	56
Dog 175						
Controls						
1	0.53	4.03	4.76		2.79	2.92
2	0.33	4.59	4.88		4.10	2.83
3	0.03	4.87	6.52		3.66	1.91
4	0.01	3.05	3.42		3.07	2.41
5	1.66	4.08	2.03		2.85	2.27
Mean	0.51	4.12	4.32		3.29	2.47
0.9% NaCl in the duodenal bulb during the 1st hr.						
1	0.02	**2.90**	3.30		4.09	3.89
2	0.19	**2.93**	4.11		2.10	2.02
3	0.09	**2.92**	1.87		2.42	—
4	0.30	**2.61**	2.84		3.47	2.54
5	0.28	**3.58**	2.47		2.24	3.82
Mean	0.18	**2.99**	2.92		2.86	3.07
In per cent of mean for controls	—	73	68		87	124
0.1 N HCl in the duodenal bulb during the 1st hr.						
1	0.08	**0.55**	1.05		3.86	3.39
2	0.95	**1.82**	1.76		1.33	1.99
3	0.92	**1.01**	2.04		2.36	2.96
4	0.76	**0.60**	0.86		0.97	1.54
Mean	0.68	**1.00**	1.43		2.13	2.47
In per cent of mean for controls	—	25	33		65	100

* Bold face figures represent instillation of NaCl or HCl into the duodenal bulb.

TABLE 1

Secretory responses to test meal in Pavlov pouch dogs with and without concomitant perfusion of the duodenal bulb with NaCl and HCl

Procedure and Experiment No.	Secretion (mEq. Total Acid)					
	Control period (1 hr.)	Response to test meal				
		1st hr.	2nd hr.		3rd hr.	4th hr.
			½ hr.	½ hr.		
Dog 148						
Controls						
1	1.10	4.29	2.09	1.80	2.08	1.52
2	0.35	3.43	1.52	1.84	2.08	0.72
3	0.46	4.39	2.09	1.97	2.29	0.75
4	0.17	3.17	1.80	1.31	2.20	0.70
5	0.18	2.51	1.57	0.90	1.32	0.97
Mean	0.45	3.56	1.81	1.57	1.99	0.93
0.9% NaCl in the duodenal bulb during the 1st and half of the 2nd hr.						
1	0.05	2.45	1.52	1.30	1.21	0.65
2	0.01	3.30	1.58	1.56	2.01	1.83
3	0.02	3.07	2.07	1.80	2.81	1.45
4	0	2.21	1.49	1.10	0.65	0.50
Mean	0.02	2.76	1.67	1.43	1.67	1.11
In per cent of mean for controls	—	78	92	91	84	119
0.1 N HCl in the duodenal bulb during the 1st and half of the 2nd hr.						
1	0.10	1.03	0.50	0.63	0.91	0.62
2	0.01	1.23	0.67	0.81	2.04	1.87
3	0.03	0.80	0.84	0.49	1.78	2.05
4	0.01	0.82	0.29	0.17	0.82	1.51
Mean	0.04	0.97	0.58	0.53	1.39	1.51
In per cent of mean for controls	—	27	32	34	70	162
Dog 165						
Controls						
1	0.48	3.49	1.72	1.96	3.97	4.11
2	0.37	4.63	3.16	2.13	3.72	—
3	1.06	3.44	2.08	2.01	4.47	3.97
4	0.56	5.31	2.83	1.98	4.37	4.31
Mean	0.62	4.22	2.45	2.02	4.13	4.31

secretion in a Pavlov pouch. The pH of the duodenal perfusate, measured as it flowed from the bulb, varied in the present experiments between 1.3 and 1.6. The pH range within which the inhibitory mechanism of the bulb is activated is not yet exactly known. Preliminary observations indicate that depression of the pH of the bulb to about 2 suffices to activate the inhibitory mechanism.[9] In our opinion it is not unreasonable to assume that under normal postprandial conditions such a degree of acidity may be reached in the duodenal bulb. Provided there is no regurgitation of buffering duodenal contents into the duodenal bulb, postprandial secretion from an empty stomach will no doubt depress the pH of the bulb to the low values capable of activating the inhibitory mechanism. If so, the physiologic role of the bulbar inhibitory mechanism may be to arrest the postprandial gastric secretion and suppress gastric acid secretion during the interdigestive periods.

For substantiation of this hypothesis, further data are required concerning the postprandial acidity in the duodenal bulb and the pH-dependence of the bulbar inhibitory mechanism.

Previous observations by Shay *et al.*[10] suggest that in patients with duodenal ulcer the hypersecretion of HCl is due to a defective duodenal inhibitory mechanism. This idea has not been generally accepted. In view of the present results the role of the duodenal inhibitory mechanism in human physiology and pathology should be reappraised.

Summary

In Pavlov pouch dogs the duodenal bulb (the proximal 3 to 4 cm. of the duodenum) was isolated from the stomach by a double mucosal wall at the pylorus. The duodenum was transected just proximal to the entrance of the common bile duct. A cutaneous fistula to the bulb was then constructed with the aid of a loop of the terminal ileum. The gastrointestinal continuity was reestablished by gastrojejunostomy. Acidification of the bulb by perfusion with 0.1 N hydrochloric acid produced a substantial inhibition of the secretory response to a test meal. The reduction averaged 75 per cent. These findings indicate that the pH-sensitive inhibitory mechanism of the duodenum is, at least in part, within the bulb. It is suggested that the physiologic significance of the mechanism lies in the suppression of gastric acid secretion during interdigestive periods.

Acknowledgment. Magnus Bergvalls Stiftelse and Svenska Sällskapet för Medicinsk Forskning have given financial support for this investigation, which is hereby gratefully acknowledged.

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Baker-'52
Bib No. 21

[illegible] CASES IN WHICH HEPATITIS DEVELOPED

Pyloric stenosis secondary to ingestion 2 cases in which hepatitis developed.

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and

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Ingestion of concentrated mineral (hydrochloric and sulfuric) acid frequently results in severe damage to the stomach but only minor and infrequent damage to the esophagus. This is surprising since the general conception is that corrosive ingestion leads predominantly to esophageal injury and stenosis. This is true of concentrated alkalis but apparently not for concentrated acids. This report of two cases with almost complete pyloric obstruction without permanent esophageal injury is prompted by the paucity of such reports in the English literature, by the excellent results following gastroenterostomy, and by the occurrence of homologous serum hepatitis after surgical convalescence.

CASE 1.—A 26-year-old white salesman was admitted to the hospital 45 days after the ingestion of muriatic acid. On that occasion the patient was inebriated and he ingested a small amount of acid in the mistaken belief that it was whiskey. He immediately spat the fluid out, induced vomiting, and then drank a glass of milk. Except for a slight "rawness in the throat," he felt well until the following day when he noted nausea and vomiting after the ingestion of food or liquids. This continued for eight days, and he was hospitalized elsewhere after two episodes of hematemesis. Supportive therapy was instituted with good results, and the patient was discharged without a diagnostic check-up. A similar episode with hospitalization occurred one week later. The third episode of nausea and vomiting without epigastric distress, but this time with dysphagia, occurred four days before admission to the hospital. The patient stated that he had lost 50 lb. (22.7 kg.) in weight in this 45 day period.

The physical examination on admission was essentially negative except for evidence of recent weight loss, moderate dehydration, and tachycardia of 120 beats per minute. The initial hemogram showed only slight leukocytosis with a normal differential count. Urinalysis was essentially negative. Blood chemistry determinations, including non-protein-nitrogen, blood urea nitrogen, blood chlorides, carbon dioxide combining power, and total proteins with the albumin globulin ratio, were within normal limits. Histamine fractional analysis of gastric contents on two occasions failed to show any free hydrochloric acid. The upper gastrointestinal x-ray series, done soon after admission, was reported as follows: "Examination of the esophagus with both thick and thin barium revealed some delay in the passage of the barium through the cardiac sphincter. However, there is no appreciable dilatation of the esophagus. The barium passes into the stomach which does not fill out and repeated attempts to secure filling of the stomach were not successful as the patient was not able to tolerate the barium. Barium remained suspended within what appeared to be the fundus of the stomach; however, there was some slight irregularity in the region of the fundus just above the suspended barium. Radiographs confirm the fluoroscopic findings and reveal a normal esophagus (fig. 1). There is a small amount of barium within the stomach; however, at no time is the barium seen beyond the incisura angularis (fig. 2). Fluoroscopic examination performed at 30 minute intervals revealed no further information. At the end of twenty-four hours, there is a small gastric residue. However, no barium is seen within the small bowel or colon (fig. 3). Impression: Findings suggest a neoplastic lesion involving the pyloric region of the stomach; advise gastroscopy."

Esophagoscopy was done and the esophagus found normal to the cardiac sphincter. Gastroscopy was performed by one of us (M. A. S.). The gastric cavity was found to be very small, holding one cupful of air. The distal portion of the stomach and the fundus were seen practically without moving the instrument, and the mucosa was intact throughout without evident ulceration or scarring. The normal rugal pattern of the stomach was not seen. Cords of tissue covered by epithelium were noted running in various directions. In some areas there was an appearance of webbing, which in some ways mimicked the cardiac chordae tendineae. In the distal portion of the visualized gastric cavity some mucus was seen, and there was a suggestion of an opening into another cavity; however, the gastroscope could not be inserted further. The impression was that there was no tumor. A congenital anomaly of the gastric rugae and mucosa was considered as a possible explanation for the peculiar findings. It was difficult to conceive that a small amount of hydrochloric acid could produce such marked and bizarre changes.

Conservative management failed to relieve the obstruction. An exploratory laparotomy was performed and the following findings noted. The stomach was diffusely thickened and scarred. The distal end of the stomach was entirely obstructed, all mucosal surfaces being in intimate contact. A posterior gastroenterostomy was then performed. A biopsy of the stomach wall, taken during the laparotomy, was later reported as showing chronic inflammation and fibrosis (fig. 4). The patient made an uneventful convalescence and was discharged two weeks later.

This patient was readmitted to the hospital six weeks later with complaints of jaundice of two days duration. This appeared to be a hepatitis of the homologous serum type, which cleared up completely with the standard high protein-high carbohydrate diet bed rest, and added vitamins and lipotropic factors. The patient had received 3 units (900 cc.) of plasma on the day preceding the exploratory laparotomy.

CASE 2.—A 49-year-old white man entered the hospital 46 days after the ingestion of commercial hydrochloric acid (muriatic acid). At that time, the patient had been mentally depressed and despondent about his employment and had ingested the acid with suicidal intent. This was done in the morning before breakfast. Shortly afterwards, he vomited copious amounts of blood, and, several hours later, his stomach was lavaged. Thereafter, his appetite remained poor, with abdominal distention and vomiting occurring after the ingestion of food. There was no dysphagia, and the patient lost over 20 lb. (9.1 kg.) in weight in the following six weeks.

On admission to the hospital, physical examination revealed a depressed, pale, slightly emaciated man whose oral mucous membranes were dry. Blood pressure was 114/80 mm. Hg, and pulse, 80 and regular. The abdomen was scaphoid, and moderate epigastric tenderness was noted. No gastric peristalsis was visible. Throughout this patient's hospital course he ate very little and vomited undigested food several times a day.

The hemogram revealed mild anemia with a red blood cell count of 3,900,000 cells per cubic millimeter and a hemoglobin count of 11.1 gm. (photoelectric colorimeter). Leukocyte count was normal. The blood chlorides, carbon dioxide combining power, urea nitrogen, and fasting sugar were all within normal limits. Total protein was 7.6 gm. per 100 cc., with albumin 3.3 and globulin 4.3 gm. per 100 cc. Liver function tests, including serum bilirubin, total cholesterol and free cholesterol ester ratio, thymol turbidity, thymol flocculation, cephalin-cholesterol flocculation, and alkaline phosphatase were all within

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normal limits. Serum potassium was 3.7 mEq. per liter. The upper gastrointestinal x-ray series was reported as follows: "Some fluid was present in the stomach. The antrum and pylorus were narrowed and elongated, producing almost complete obstruction. The narrowing was symmetrical (fig. 5 and 6). At four hours, very little barium had left the stomach. Impression: Infil-

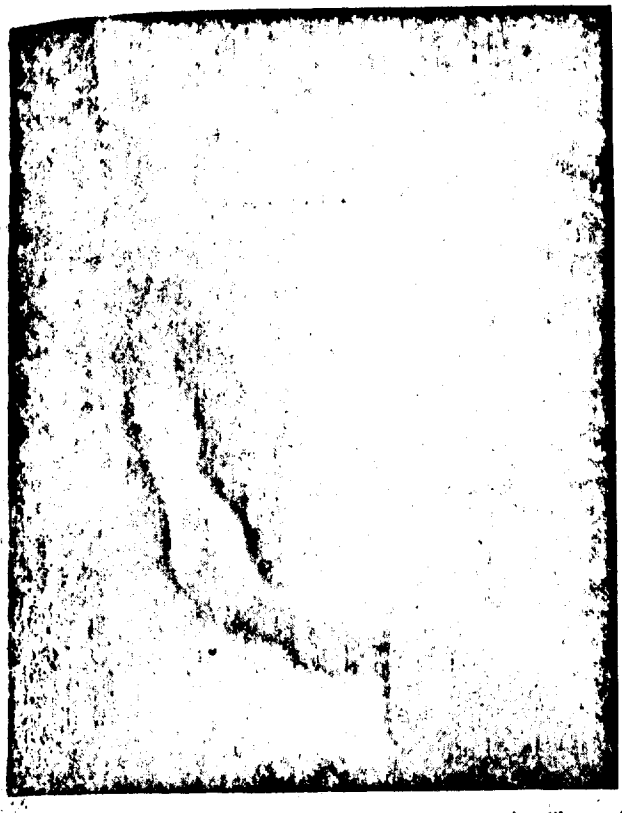

Fig. 1 (case 1).—Barium-filled esophagus showing normal caliber and distance of obstruction at cardia.

trative neoplasm involving the antrum and pylorus." Gastric aspirations 24 hours later revealed barium in the gastric contents in spite of repeated emesis. A histamine fractional gastric analysis failed to reveal any free hydrochloric acid.

Subsequently, gastroscopy was performed by one of us (M. A. S.). The instrument was inserted through the esophagus with ease but became arrested in the fundus. Retained food particles were noted. The mucosa was extremely hyperemic and slightly friable. Rugal folds ran radially rather than parallel, suggesting cicatrization. The instrument could not be inserted into the antral region. The impression was a marked superficial gastritis with pyloric obstruction and cascade stomach, or changes in shape due to cicatrization. Because of poor response to conservative therapy, an exploratory laparotomy was done about two weeks after admission. At surgery, cicatrization of the antrum and pylorus were noted. The stomach could not be well mobilized. A posterior gastroenterostomy was performed. The patient had an uneventful convalescence, with rapid gain in weight; however, 50 days after surgery, icterus and a slightly enlarged, tender liver were noted, and a diagnosis of homologous serum hepatitis was made. The patient had received 500 cc. of blood at the time of surgery. Subsequent convalescence was uneventful.

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COMMENT

The gastric sequalae of corrosive poisoning were fairly well summarized by Boikan and Singer in 1930.[1] It has been pointed out by these workers as well as others[2] that little attention has been paid to this entity in the English literature. Although pyloric stenosis following the ingestion of corrosive acid was described clinically and pathologically about 125 years ago, a recent review[2a] quotes only 3 references in English among a total of 17.

It is well established that alkaline corrosives when swallowed frequently cause extensive necrosis of the esophagus with later cicatricial changes, but because of neutralization in the stomach they cause little damage there; thus, Heindl[3] found that only 20% of 116 cases of esophageal strictures due to lye had associated pyloric stenosis. On the other hand, inorganic acids, in their rapid passage through the esophagus, cause slight damage to this structure, while the prolonged contact with the gastric mucosa due to associated pylorospasm leads to extensive damage to the stomach. Thus, of 86 cases with late pyloric stenosis after acid ingestion,[1] only 20% had associated esophageal stenosis; however, individual reports since these reviews have noted esophageal stenosis in only 2 of 30 cases of late pyloric stenosis (6.5%). Besides the inorganic mineral acids, formaldehyde has been reported to cause gastric stricture without esophageal stenosis,[5] and recently isolated pyloric obstruction secondary to lye ingestion is reported.[2b]

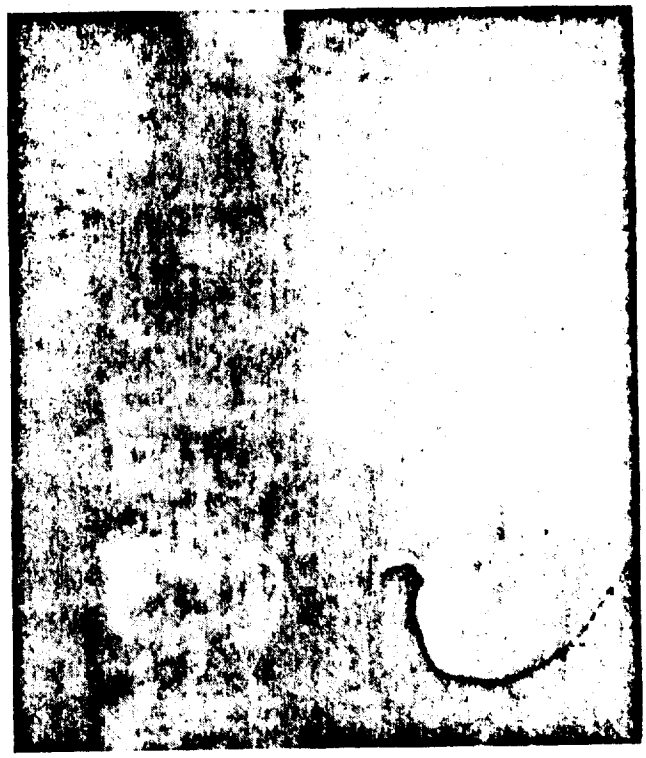

Fig. 2 (case 1).—Roentgenogram of stomach showing antral narrowing and pyloric stenosis.

In the consideration of the factors affecting location and type of gastric lesions produced, not only the type of corrosive ingested but also its concentration are important. The highly concentrated poisons frequently cause perforation and early death. Sick[6] reported a case of

pyloric obstruction following the ingestion of a small amount of dilute acid and, in addition, experimentally demonstrated the importance of a small amount of dilute corrosive in producing extensive gastric damage.

The state of the stomach at the time of corrosive ingestion has been thought to affect the nature of the gastric lesion produced. In a full stomach, the fluid passes quickly to the pylorus, where it is arrested by pylorospasm and spreads out in triangular fashion with the apex toward the cardia and the base ending just proximal to the pyloric ring. This pathological picture was noted in one of Boikan and Singer's cases at autopsy two weeks after the ingestion of dilute lye. If the validity of the "magenstrasse" is accepted, then the corrosive contacts the lesser curvature and pyloric mucosa on the one side and the diluting effects of food and or liquid on the other. Emesis then leaves a raw ulcerated lesser curvature and pars pylorica. On the other hand, in the empty contracted stomach in which the lesser curvature tends to attain a vertical position, the corrosive contacts the entire mucosa with longest contact in the pars pylorica and media. In this situation, the corrosive is bordered by mucosa of a spastic pylorus distally and the fundic air, above the fluid, proximally. Thus here, late sequalae are

Fig. 3 (case 1).—Roentgenogram of stomach 24 hours after ingestion of barium meal still showing retention.

pyloroantral constriction with simultaneous narrowing of the pars pylorica and pars media or total gastric scarring (linitis plastica type), or hourglass stenosis due to antral constriction. In both instances, the prepyloric area carries the brunt of the damage. We feel that the first of our cases (case 1) probably represents the "linitis plastica" type of total gastric scarring, and the second (case 2) is an example of the pyloroantral constriction type; however, the types of lesions found in our patients tend to refute the accepted relation between gastric con-

Fig. 4 (case 1).—Biopsy of stomach wall showing fibrosis and round cell infiltration.

tent and resultant gastric damage. Patient 1 had been on a drinking spree at the time of corrosive ingestion, and his stomach had been presumably, at least partially full, and yet the lesion was diffuse. It might be explained that the induced emesis produced contact between the corrosive and the mucosa of the upper half of the stomach with the production of the diffuse scarring as noted later at laparotomy. Patient 2 ingested the corrosive in the morning before breakfast on a presumably empty stomach, and the cicatrization was chiefly antral and pyloric.

In addition to stenotic lesions, other sequalae ascribed to hydrochloric acid ingestion are acute corrosive gastritis and acute emphysematous gastritis.[7] Both Schindler[8] and Palmer[9] have described the gastroscopic picture of acute corrosive gastritis with subsequent return of a normal gastroscopic picture. The latter author noted the return of a gastroscopically normal stomach by the 55th day and felt that this type of evidence refuted the idea of a "magenstrasse." He explained the late pyloric involvement on the basis of "the greater susceptibility of the antral region to noxious influences" rather than on the basis of greatest concentration of corrosive.

7. Weems, H. S.: Emphysematous Gastritis, Am. J. Roentgenol. 56: 588, 1946.

8. Schindler, R.: Gastritis, New York, Grune & Stratton, Inc., 1947, p. 411.

9. Palmer, E. D., and Scott, S. M., Jr.: Acute Corrosive Gastritis: Observations on the Gastric Mucosa Following Ingestion of Concentrated Hydrochloric Acid, Gastroenterology, 12: 579, 1949.

In the English literature, Putnam [10] reported a case of pyloric excision for stricture secondary to acid ingestion in 1915. McLanahan [11] in 1934 and Arena [12] in 1936 reported cases of pyloric obstruction following muriatic acid ingestion. In these cases, as in ours, the pyloric stenosis became markedly symptomatic in 3 to 6 weeks. Boikan and Singer [1] felt that late gastric stenosis should be anticipated in all cases of corrosive ingestion, particularly with acids. Schulenburg [13] reported a case and based his conclusions for local damage on the experimental work of Testa with barium-caustic soda studies in dogs.

In the convalescent course of both the patients reported, hepatitis, presumably of the homologous serum type, was discovered about six weeks postoperatively. Neither patient had any previous evidence of gross liver damage, although the blood globulin concentration in case 2 was slightly elevated. In both cases, the disease process was relatively mild, and both patients recovered without residua. One may speculate about the possible explanations for the hepatitis in both of these cases. The patients were treated in different hospitals but received blood and plasma from the same bank. The first patient received one blood transfusion of 500 cc. The following

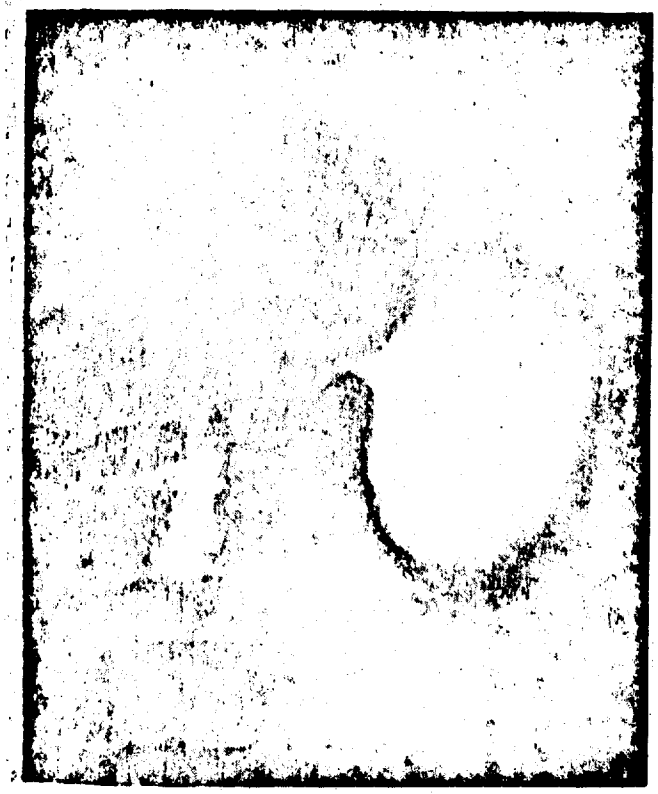

Fig. 5 (case 2).—Roentgenogram of stomach showing marked contraction of antrum and pyloric stenosis.

possibilities exist. 1. It was entirely coincidental that hepatitis developed in both of these patients with the same illness. 2. The lowered resistance and malnutrition of these persons made them an easy prey to this infection. 3. The specific damage and extensive erosions of the gastric mucosa may predispose to viral hepatitis. 4. This particular type of gastric injury may result in hepatitis independent of the use of blood or plasma. While the last two possibilities are highly speculative, this complication should be anticipated and reported in the future to further elucidate these points.

TREATMENT

Therapy during the acute phase is supportive, including measures to combat infection, hemorrhage, and/or shock. Neutralization with bicarbonate or other gas re-

Fig. 6 (case 2).—Roentgenogram of stomach six hours after ingestion of barium showing practically no emptying.

leasing substances should be avoided. Lavage should be undertaken with extreme caution because of a tendency of the stomach to perforate. Elisher [14] noted that perforation and collapse was less frequent after hydrochloric acid ingestion than after sulfuric acid ingestion. Fluid balance and nutrition should be maintained by parenteral fluid and blood transfusions. The latter should not be used indiscriminately in view of the hepatitis noted in our two cases. Meyer and Steigmann [15] emphasized the edema and friability of the stomach during the acute phase, precluding suturing of the gastric wall. The recommended jejunostomy at this stage if necessary.

Later therapy is directed toward the late sequelae, i. e., pyloric obstruction. Gray and Holmes [2a] recommended partial gastrectomy in young patients in view of the possibility of malignant degeneration, stomal ulcer for-

10. Putnam, C. R. L.: Excision of the Pylorus for Stricture due to the Ingestion of an Acid, M. Rec. 87: 332, 1915.
11. McLanahan, S.: Pyloric Occlusion Following the Ingestion of Corrosive Liquids, J. A. M. A. 102: 735 (March 10) 1934.
12. Arena, J. M.: Pyloric Stricture Following Ingestion of Muriatic Acid, South. M. J. 29: 331, 1936.
13. Schulenburg, C. A. R.: Corrosive Stricture of Stomach Without Involvement of Oesophagus, Lancet 2: 367, 1941.
14. Elisher, E., quoted by Gray and Holmes.[2a]
15. Meyer, K. A., and Steigmann, F.: The Surgical Treatment of Corrosive Gastritis, Surg., Gynec. & Obst. 79: 306, 1944.

mation, and/or the difficulty of accomplishing satisfactory gastroenterostomy. The last mentioned would seem most plausible while the former two are merely conjectural. Gradman and his colleagues feel that gastroenterostomy is the simplest and safest procedure, providing that the gastric wall at the anastomotic site is normal. We agree with this completely. In our two cases posterior gastrojejunostomy yielded excellent results. If a direct anastomosis is not feasible, then the procedure of choice is a subtotal gastric resection.

SUMMARY

Two cases of stenotic gastric lesions without esophageal involvement secondary to commercial hydrochloric acid ingestion are reported, and attention is called to the paucity of such reports in the English literature.

The relation of the quantity of gastric contents to the type and nature of gastric lesions produced is discussed, and the occurrence of postoperative hepatitis in both cases is noted.

109 N. Wabash Ave. (Dr. Spellberg).

Casopis Lekaru Ceskych, No. 29, 893-895 (1973) 616.334-007.271-053.2

STENOSIS OF THE PYLORUS IN A CHILD AFTER INGESTION OF HYDROCHLORIC ACID *)

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Contribution from the Internal Pediatrics Division (Acting Head Dr.E. Kratkova) and from the Division of Pediatric Surgery and Orthopedics (Acting Head,Assistant (Lecturer or Reader) Dr.V.Tosovsky),S.F.N.(State Fund Hospital),Prague

The ingestion of corrosive solutions during childhood is a fairly common occurrence.. In most cases, soda caustic is involved, whereas acids are involved only very seldom (as well as other solutions). These incidents, whose immediate effects will not be discussed here, usually result in the ulcerous stenosis of the esophagus. These cases are common also in our divisions and the associated problematic has been frequently reviewed (Mikula).

Previously,however, we did not observe in our Divisions the generation of the stenosis of pylorus after subjection to the action of a corrosive agent. The female patient, whom we shall discuss, represents the first case of this type, which is interesting due to the fact that the ingestion of hydrochloric acid left the esophagus intact and led to the damage of only the pyloric part of the stomach.

The following information is cited from the case history Record No.5253/50: A girl, 16 months old, was transported on May 30, 1950 from a hospital at the border to the II. Pediatric Division of Internal Medicine of S.F.N. (State Fund Hospital). The patient has no siblings. The birth was normal, the birth weight was 3600 g. The child was nursed for only 11 days. It did not receive vitamins. Prior to this time, it never moaned or vomited. At home, due to negligence, the child reportedly ingested an unknown amount (probably very small) of hydrochloric acid. The child was treated for fourteen days at the rural hospital and discharged without apparent complications following a negative esophagogram. Soon after discharge from the hospital, the child started

vomiting. It vomited every type of ingested food, it had few stools and urinated relatively little. In 14 days, it lost almost 2 kg in weight.

Findings at the admission time: A small girl with a graceful build, of the asthenic type, neuropathic, very disagreeable and tearful. Weight, 8.75 kg. She is fairly well supplied with blood, but the skin turgor is very depressed. The large fontanel is still open. The conjunctivasare considerably blood-shot. The mucous membranes of the oral cavity are bright red, the gums are swollen, and the tongue is coated. The mucous membrane of the pharynx is red and without any coatings or visible sign of a necrosis. Pea-sized lymph glands on the nect and signs of passed rachitis on the chest are noted. Lungs and heart are without pathological changes. The abdomen is at the same level as the chest, breathes over its entire area, its walls are soft, whereas the percussion and palpation examination gave normal findings. The urine contains a trace of albumin. Sugar, bile pigments, and acetone were not detected. The urine sediment contained only isolated leucocytes. The blood analysis indicated leucocytosis, light anemia, and considerable shift toward the left. The tuberculin, BWR, and agglutination tests were negative, whereas the S.E. result was increased (24 mm/hour). The residual nitrogen was slightly increased, the cholesterol and protein levels in the blood were considerably increased, whereas the chlorides were within normal limits.

The child is continually thirsty, it refuses solid food, but eagerly reaches for beverages. Following the consumption of a considerable amount of fluids, it explosively vomits copious quantities of mucous brownish material. On the third day after admission it has a small quantity of thick stool. During its stay in the Division, the spastic vomiting increases in intensity as well as frequency. The child is maintained on repeated infusions of glucose and of the physiological solution containing vitamins. A tightening of the stomach walls was not observed and a tumor of the pylorus could not be detected

by palpation.

A direct laryngoscopic and esophagoscopic investigation (Prof.Dr.Wiskovsky) indicated only considerable blood oversupply of the mucous membranes without any appreciable narrowing of the esophagus.

During the x-ray investigation, the contrast fluid freely and rapidly proceeds to the stomach from the esophagus. The stomach is hook-shaped ectatic, and extends to the small cavity. The peristaltic of the walls is lively and extends to the pylorus. The contrast fluid does not proceed through the pylorus even after 24 hours.

Thus, a complete closure of the pylorus was clearly indicated and, therefore, after intensive internal treatment (panicillin, continuous dropping infusion and transfusions), we commenced an operation, which was performed on June 8, 1950 at the Division of Pediatric Surgery and Orthopedics of S.F.N. (State Fund Hospital). (Docent(Associate Professor) Dr. Kafka).

With local anesthesia,we opened the abdominal cavity with a central section. The stomach is ectatic, thick-walled, and the pyloric region exhibits hyperemic serostis. The pylorus is deformed and narrowed over a 2-3 cm section. In this location, there is a palpable oblong, ulcerous formation, which is located deeply in the mucous membrane and is fixed to the anterior muscular layer. By a tactile investigation of the stomach wall, it is possible to locate a circular, sharply limited stenosis. Due to the serious condition of the patient, we decided that a resection of the stomach would be too demanding and therefore, we began a posterior gastro-jejunostomy.

After a 4-day continuous application of dropping infusion and of repeated transfusions, we overcame the serious postoperative condition. Due to fever, we continued the penicillin administration for 14 days after the operation (Gingivitis and stomatitis). After a careful realimentation,a thin stool was obtained after three days. Vomiting appeared once during the second day following the operation. On the seventh day, all stitches were removed, the

wound being healed per primam. The appetite toward food continuously increased and the child was discharged into home care after three weeks, during which time it gained 1 kg in weight, and all complaints ceased. An examination 6 weeks later showed that the child gained an additional 2 kg and that it accepts normal food well. Further examination proved impossible because the child was not brought in by the parents in spite of repeated notices.

In approaching the operation, which was unavoidable from the point of view, we found ourselves in doubt concerning the correct diagnosis because the findings in the oral cavity and in the esophagus did not show the effect of corrosive attack and the anamnestic indications had to be accepted with a certain amount of reserve as to reliability. The x-ray examination merely indicated to us the existence of the stenosis of the pylorus. It was possible that in the case of our patient, there could have been a mistake in the anamnesis, or that the patient's condition could have been a chance complication (arising from the ingestion of acid) in addition to some basic, heretofore clinically unmanifested, illness. Roughly the following possibilities could be considered (Podlaha, Steep, Huber, et al.):

1. Delayed pylorostenosis 2. Ulcerogenous narrowing of the stomach outlet 3. Changes in the adjacent region compressing the stomach outlet 4. Abscess 5. Nervous disturbances generating stomach outlet craps 6. Stenosis following corrosive attack.

On 1. We excluded the delayed pylorostenosis because it has a different character as to generation and development and because it would have manifested itself earlier. Although several cases have been described in the literature, e.g., the Crystalli patient was operated on at an age of 14 years, we still consider it as controversial that the pylorostenosis of infants may be included in the nosologic concept.

On 2. Gastroduodenal damage is rare in infancy, but as indicated by the literature, and as we have seen ourselves, this type of damage is observed in

infants also, as well as even in newly borne. Damages of the pylorus are, however, relatively rare. Although we could not exclude an ulcerous structure as the basis for the damage altogether, the case history lacked the typical criteria of a prior disease, such as pains, bleeding, or other long-standing symptoms.

To 3 and 4 The relatively sudden appearance of pyloric closure against stenoses based on changes in the vicinity of the stomach outlet, such as strangling caused by connecting tissues, membranes, postinflammation growths, or such as compression of the stomach outlet due to swelling of the mesenterial nodes, due to the luetic, or other stenoses, and, finally, due to abscesses. Findings indicating the presence of any of the above conditions were lacking in the case of our patient.

To 5 A stomach outlet closure of the cramp type may accompany all of the above-mentioned ailments, but may also appear independently. This condition is controllable, since it may be treated with spasmolytics.

To 6 Due to the above-mentioned considerations, we leaned toward the point of view that an ulcerous stenesis may result from the corrosive attack by acid. We were led toward this view by the anamnesis, as well as by the case history, which may be regarded as typical after reviewing the corresponding literature references.

The ingestion of corrosive materials is not a rare occurrence. Ulcerous stenoses of the esophagus are almost an every-day phenomenon in our Divisions. Stomach strictures, however, with a concurrent esophagus damage, are rare. In addition, cases are also known in which there was no esophagus damage, but the effects of corrosive attack were observed on the stomach only. This is the case after ingestion of acids, when the isolated stenosis of the pylorus (Strode, Melvin and Orator) is the usual result. Only in 6 to 20 percent of the cases there is observed a simultaneous stenosis of the esophagus. Any part of the stomach may be affected. The extent and depth of the damage is

determined by the concentration and amount of the ingested solution as well as by the state of the stomach content. It is interesting that the relatively more hazardous solutions are those that are more dilute and which are tolerated by the stomach and are not expelled, so that the exposure time of the mucous membrane to the effect of the corrosive agent is dangerously prolonged.

It is very characteristic that the stenoses resulting from acid ingestion are produced very rapidly. Often, as in our case, the patient recovers after two weeks, so that the patient is discharged from care and the pyloric stenosis (or another lesion) subsequently develops with great rapidity. The patient suddenly starts to vomit, since during the course of several days, the stomach outlet closure becomes virtually complete. This is followed by dehydration, strong alkalosis, rapid weight loss, etc.

As demonstrated by Pavlov in experiments on dogs, as well as later by Testa in 1943, the most significant damage takes place when the corrosive agent is retained by a stomach outlet cramp, i.e., in the prepyloric part of the stomach, or in the pylorus itself. It was shown in most cases that a single ulceration of the pyloric ring takes place. These data also apply to our case, which, in agreement with all literature reports exhibits all of the characteristic symptoms of corrosion by acids.

It is clear that in cases of pyloric stenosis, the treatment becomes exclusively surgical, except, of course for the acute complications, such as stomach perforation, etc.

The surgical methods for dilating the pylorus historically include resection, plastic surgery of the pylorus and creation of links bypassing the obstacle.

This second problem appeared before us during the laporotomy itself. The clarification of the etiology of the stenosis would have been certainly aided by a histoligical study and a resection of the stenotic portion, as the most

radical action, would have been therapeutically the most advantageous course and, followed by the end-to end reconstruction of the passage, also the most physiologically advantageously course as well. Of course, stomach resection in small children in general, and in those exposed to corrosive attack in particular, has its hazards. However, in the younger children, the duodenum is very fine, whereas the stomach walls are significantly hypertrophic in the case of a pyloric stenosis, so that the incongruence of these two regions is much greater than in adults. This leads to many complications in the operative technique. However, the stomach walls damaged by corrosion possess a changed consistency, are fragile and this increases the danger of stitch insufficiency, particularly in those cases where the corrosive attack is diffuse, or disperse and the diseased part cannot be removed entirely in a radical way.

The same objections may also be cited against the pyloroplastic surgery, although this was successfully executed by Arena on a two-year old child.

Sometimes, resection is also counterindicated by the overall state of the patient, as in our case. For this reason, we selected gastrojejunostomy as the most suitable method in the present case. This method is cited as the method of choice in the literature for treating pyloric stenoses after corrosive attack.

Summary

A complete stenosis of the pylorus developed in a 16-months old child 4 weeks after hydrochloric acid ingestion. We assume that the corrosive solution generated a reflexive cramp of the pylorus, which made possible a prolonged action of hydrochloric acid on a limited area. In this way, it is possible to explain the fact that changes appeared only in the pyloric part of the stomach and led gradually to a stenosis. We suppose that the neuropathic

constitution of the child contributed to an increase of the spasm intensity.

For the therapy of the stenosis of the pylorus due to corrosive attack, only surgical treatment is appropriate. Gastro-jejunostomy is the most suitable treatment method in the majority of cases. With a correct internal preparation for the operation, with postoperative care, and with the use of antibiotics, most cases may be saved.

(Translated by Carl Demrick Associates, Inc/JP/t)

Beco-'51
Bib No. 29

Casop. Lek. Cesk. 90:893-896. 1951.

Z PRAXE

616.334-007.271-053.2

Stenosa pyloru u dítěte po požití kyseliny solné.*)

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Z II. dětského interního oddělení (zast. přednosta Dr E. Kratková) a z oddělení pro dětskou chirurgii a orthopedii (zast. přednosta as. Dr V. Tošovský) S. F. N. v Praze.

Požití leptavých roztoků v dětském věku je dosti častou příhodou. Většinou jde o louh, vzácně o kyseliny (či jiné roztoky). Tyto příhody, jejichž bezprostředními následky se nebudeme zabývat, vyústí obyčejně v jizevnatou stenosu jícnu. Jsou běžné i na našich odděleních a jejich problematika byla často přetřásána. (Mikula.)

*) Předneseno s demonstrací případu na schůzi SČL dne 9. X. 1950.

Dosud však nikdy jsme nepozorovali na našich odděleních vznik stenosy pyloru po poleptání. Nemocná, kterou demonstrujeme, je náš prvý případ toho druhu, zajímavý tím, že po požití kyseliny solné jícen zůstal intaktní a byla poškozena isolovaně pouze pylorická část žaludku.

Z chorobopisu č. prot. 5253/50 uvádíme: Děvče, staré 16 měsíců, bylo přivezeno 30. V. 1950 z pohraniční nemocnice na II. dět. odd. int. S. F. N. Rodiče dítěte jsou zdrávi. Sourozence nemá. Porod byl normální, porodní váha 3600 g. Kojeno bylo jen 11 dní. Vitaminy nedostávalo. Dosud nikdy nestonalo, ani nezvracívalo. Doma před měsícem nedopatřením vypilo prý neznámé množství (pravděpodobně velmi malé) kyseliny solné. Čtrnáct dní bylo léčeno ve venkovské nemocnici a zdánlivě bez obtíží propuštěno domů — při negativním nálezu na oesofagogramu. Brzy po propuštění z nemocnice začalo zvracet. Zvracelo každou požitou potravu, mělo málo stolic a poměrně málo močilo. Za 14 dní ubylo na váze skoro 2 kg.

Nález při přijetí: děvčátko gracilní kostry, asthenického typu, neuropathické, velmi mrzuté a plačtivé. Váha 8,75 kg. Kůže je dosti dobře prokrvená, turgor kožní velmi poklesly. Velká fontanella dosud neuzavřená. Spojivky značně překrvené. Sliznice dutiny ústní jasně červené, dásně zduřelé, jazyk je povleklý. Sliznice faryngu rudá, bez povlaků a viditelných známek nekrosy. Na krku hráškovité mízní uzliny. Známky proběhlé rachitidy na hrudníku. Plíce a srdce bez pathol. změn. Břicho v rovině hrudníku, dýchá v celém rozsahu, jeho stěny jsou měkké, poklepový i pohmatový nález v mezích normy. Meningeální příznaky nepřítomny. V moči stopa bílkoviny. Cukr, žlučová barviva ani aceton nebyly zjištěny. V močovém sedimentu pouze ojedinělé leukocyty. V krevním obraze: leukocytosa, lehká anemie a značný posun doleva. Zkoušky tuberkulinové, BWR i aglutinace negat. S. E. zvýšená (24 mm/1 hod.). Zbytkový dusík lehce zvýšený, hladiny cholesterolu i bílkovin v krvi značně zvýšené, chloridy v normál. mezích.

Dítě je stále žíznivé, samo odmítá hustou stravu, ale dychtivě sahá po nápoji. Po požití většího množství tekutin explosivně zvrací bohatý, hlenovitý a zahnědlý obsah. Třetí den po přijetí má nehojnou, hustou stolici. Během pobytu na oddělení spastické zvracení nabývá na intensitě a je častější. Dítě je udržováno opakovanými infusemi glukosy a fysiologického roztoku s vitaminy. Ztužování stěn žaludku jsme nepozorovali a tumor pyloru jsme nenahmatali.

Direktní laryngoskopické a oesofagoskopické vyšetření (prof. Dr. Wiškovský) ukazuje pouze značné překrvení sliznic bez patrného zúžení jícnu.

Při roentg. vyšetření náplň kontrastní volně a rychle prochází jícnem do žaludku. Žaludek je hákovitého tvaru, ektatický, sahá až do malé pánve. Peristaltika stěn je živá a pokračuje až do pyloru. Pylorem ani po 24 hod. náplň neproniká.

Rozpoznání úplného uzávěru pyloru bylo tedy jasné, a proto po intensivní interní léčbě (penicilin, stálé kapací infuse a transfuse) jsme přistoupili k operaci, která byla provedena dne 8. VI. 1950 [illegible] odděl. pro dětskou chirurgii a orthopedii S. F. [illegible] (doc. Dr Kafka).

V místním znecitlivění jsme otevřeli středním řezem dutinu břišní. Žaludek je ektatický, tlustostěnný, v oblasti prepylorické je serosa hyperemická. Pylorus je deformován a zúžen v úseku 2—3 cm. Zde je hmatný podélný jizevnatý útvar, který je uložen v hloubi — na sliznici — a je fixován k přední svalové vrstvě. Při vyšetření oblasti pyloru vchlípením stěny žaludeční lze zjistit cirkulární ostře ohraničenou stenosu. Vzhledem k těžkému stavu nemocné jsme usoudili, že resekce žaludku by byla příliš náročná a založili jsme zadní gastro-jejunostomii.

Po 4denní aplikaci stálé kapací infuse a opakovaných transfusích jsme zvládli těžký pooperační stav. Pro horečnatý stav jsme pokračovali 14 dní po operaci v podávání penicilinu (Gingivitis a stomatitis). Při opatrné realimentaci byla třetího dne první řídká stolice. Zvracení se objevilo jednou, druhý den po operaci. Sedmý den byly vyňaty stehy, rána se zhojila per primam. Chuť k jídlu stále vzrůstala a dítě po 1 kg přírůstku na váze bylo propuštěno bez obtíží po třech týdnech domů. Při kontrole za 6 týdnů dobře prospívá, přibralo další 2 kg a dobře snáší normální stravu. Další kontrola nebyla možná, jelikož se rodiče s dítětem nedostavili přes dvojí naši výzvu.

Přistupujíce k operativnímu výkonu, který byl s hlediska klinického nevyhnutelný, byli jsme ve značných diferenciálně diagnostických rozpacích, a to již vzhledem k tomu, že nález v dutině ústní i na jícnu neprokázal stopy po poleptání a že údaje anamnestické co do spolehlivosti bylo možno brát s jistou reservou. Roentgenové vyšetření nás poučilo jen o přítomnosti stenosy pyloru. Mohlo tudíž u naší nemocné jít buď o omyl v anamnese, nebo o náhodnou komplikaci (požití kyseliny) při základní, dosud klinicky se neprojevivší nemoci. Bylo možno pomýšlet zhruba na tyto možnosti (Podlaha, Steep, Huber a j.):

1. Pozdní pylorostenosa. — 2. Ulcerogenní zúžení vratníku. — 3. Změny v okolí stlačující vratník. — 4. Nádor. — 5. Nervové poruchy vyvolávající křeč vratníku. — 6. Stenosa po poleptání.

Ad 1. Pozdní pylorostenosu jsme vyloučili proto, že má odlišný charakter ve vzniku i v průběhu, a byla by se patrně projevila již dříve. I když v písemnictví bylo popsáno několik případů, na př. Crystalliho nemocná operovaná ve věku 14 let, přece pokládáme za sporné, zda je lze zahrnout do nosologického pojmu pylorostenosy kojenců.

Ad 2. Vřed gastroduodenální je v dětském věku vzácný, jak však údaje z písemnictví ukazují a jak jsme sami viděli, vyskytuje se i u kojenců, dokonce i u novorozenců. Poměrně vzácnější jsou vředy pyloru. Ačkoliv jsme nemohli zcela vyloučit jizevnatou strukturu na podkladě vředu, přece nám v obrazech chyběly typické známky předchozího onemocnění, jako bolesti, krvácení a jiné dlouhodobé obtíže.

Ad 3. a 4. Poměrně náhlý vznik pylorického uzávěru mluví proti stenosám na podkladě změn v okolí

vrátníku, ať již jde o zaškrcení způsobené vazy, cévami, pozánětovými srůsty, dále stlačení vrátníku způsobené zduřelými mesenteriálními uzlinami, stenosy luetické nebo jiné, a konečně i nádory. U naší nemocné chyběly nálezy, které by svědčily o uvedené nemoci.

Ad 5. Křečový uzávěr vrátníku může provázet všechna zmíněná onemocnění, může se však objevit i samostatně. Často je pomíjivý nebo jej lze ovlivnit spasmolytiky.

Ad 6. Vzhledem k uvedeným úvahám jsme se přiklonili k názoru, že může jít o jizevnatou stenosu po poleptání kyselinou. Vedla nás k tomu jak anamnesa, tak zejména průběh onemocnění, který po pročtení příslušného písemnictví můžeme označit za typický.

Požití žíravých látek není vzácným zjevem. Jizevnaté stenosy jícnu po louhu nebo jiných alkáliích jsou dokonce zjevem všedním i na našich odděleních. Striktury žaludku se současně poškozeným jícnem zjevují se vzácně. Jsou však též známé případy, při nichž nedošlo k postižení jícnu, nýbrž se následky poleptání projevily pouze na žaludku. Stává se tak zejména po požití kyselin, kdy isolovaná stenosa pylorus (Strode, Melvin, Orator) je obvyklým následkem. Jen v 6 až 20% (Quénu a Petit) se najde současně stenosa jícnu. Postižena může být kterákoliv část žaludku. Rozsah a hloubka poškození se řídí koncentrací a množstvím požitého roztoku a stavem naplnění žaludku. Je zajímavé, že poměrně nebezpečnější jsou roztoky zředěnější, které mohou být v žaludku tolerovány a nejsou vyvrhnuty, takže doba vystavení sliznice účinku žíraviny je tím nebezpečně prodloužena.

Velmi příznačné pro stenosy po požití kyselin je rychlost jejich vzniku. Často proběhne uzdravení nemocného — jako i v našem případě — ve dvou týdnech, takže nemocný bývá propuštěn z ošetřování, načež se vyvíjí rychle pylorická stenosa (nebo jiná lacse). Nemocný začne náhle zvracet, jelikož se během několika dnů stává uzávěr vrátníku téměř úplným, dále dochází k dehydraci, silné alkalose, rychlé ztrátě na váze atd.

Jak ukázal již Pavlov v pokusech na psech a po něm v roce 1943 Testa, dochází k nejvýznačnějšímu poškození tam, kde žíravina byla zadržena křeči vrátníku, to jest v prepylorické části žaludku nebo přímo v pyloru. Bylo prokázáno v největším počtu případů jediné zjizvení na pylorickém prstenu. Tyto údaje mluví i pro náš případ, který souhlasně s údaji v písemnictví vykazuje všechny typické znaky po poleptání kyselinami.

Je zjevné, že se tam, kde dojde ke stenose pyloru, stává onemocnění výlučně chirurgickým, nehledě ovšem k akutním komplikacím, jako je perforace žaludku a pod.

Z chirurgických method, když přehlédneme operativní dilataci pyloru jako methodu patřící historii, užívá se resekce, pyloroplastiky a spojky obcházející překážku.

Před tento druhý problém jsme byli postaveni při laparotomii samotné. K objasnění etiologie stenosy pyloru by jistě přispělo histologické vyšetření a resekce stenotického úseku — jako nejradikálnější výkon — by bývala jistě therapeuticky nejvýhodnější a s následnou rekonstrukcí pasáže end to end také nejfysiologičtější. Ovšem resekce žaludku u dětí obecně a zvláště po poleptání má svá nebezpečí. Jednak u menších dětí je duodenum velmi jemné, stěny žaludku při stenose pyloru naopak značně hypertrofické, takže inkongruence těchto dvou úseků je daleko, větší než u dospělých, se všemi následnými obtížemi operační techniky. Jednak stěny žaludku poškozené leptavým roztokem mají změněnou konsistenci, jsou křehké a tím je zvýšeno nebezpečí insuficience stehu, zvláště tam, kde poleptání je difusní nebo dispersní a onemocnělou část nelze celou radikálně odstranit.

Stejné námitky lze uvést i proti pyloroplastice, ačkoliv ji Arena provedl s dobrým výsledkem u dvouročního dítěte.

Proti resekci mluví někdy též hrozivý celkový stav nemocného, jako v našem případě. Proto jsme volili gastro-jejunostomii jako nejvhodnější methodu pro daný případ, výkon, který také v písemnictví je uváděn jako methoda volby u stenos pyloru po poleptání.

Souhrn:

U 16měsíčního dítěte se vyvinula po požití kyseliny solné během 4 týdnů úplná stenosa pyloru. Předpokládáme, že leptavý roztok vyvolal reflektorickou křeč pyloru, která umožnila protrahovaný účinek kyseliny solné v ohraničeném úseku. Tak lze vysvětlit, že došlo ke změnám jen v pylorické části žaludku, vedoucím postupně ke stenose. Domníváme se, že neuropatická konstituce dítěte přispívala ke zvýšení spasmu.

V therapii stenosy pyloru po poleptání je vhodná jedině léčba chirurgická. Gastrojejunostomie je nejvhodnější methodou u většiny případů. Při správné interní přípravě k operaci, při péči pooperační a za podpory antibiotik lze dnes většinu případů zachránit.

Резюме.

У ребенка в возрасте 16 месяцев образовался полный стеноз привратника желудка в течение 4 недель после того, что он проглотил соляную кислоту. Мы полагаем, что едкий раствор вызвал рефлексную судорогу привратника, которая дала возможность продолжительного действия соляной кислоты на ограниченном участке. Таким образом можно объяснить почему описанные изменения произошли исключительно в области привратника желудка и в связи с чем это способствовало постепенно его сужение вплоть до стеноза. Мы также считаем, что невропатическая конституция ребенка благоприятствовала увеличению спазма.

Единственное подходящее лечение стеноза привратника после ожога едкой кислотой является хирургическое вмешательство. Gastrojejunostomia наиболе пригодная оперативная метода в боль-

шинстве случаев. Правильная подготовка больного перед операцией и тщательное послеоперационное попечение при содействии антибиотик даст возможность спасти главную массу описанных случаев. Сп.

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Benyo-'65
Bib No. 34

From: Med. Pharmacol. exp. 13: 317-324 (1965) (German)

From the Physiol. Lab. of the Second Surgical Clinic and the Research Laboratory for Experimental Medicine of the Medical Univerity in Budapest

The effect of duodenal acidification on the hepatic circulation (*)

By I. Benyo, Fr. Fösy, P. Görgo, G. Ludany and A. Mitsanyi

Recent test results have proved that the acidification of the duodenum causes and regulates numerous functions of the organism. These changes not only affect the micro/macro motility and secretion activity of the gastrointestinal tract, as is generally assumed, but they influence also the resorption and permeability conditions of the intestine as well as the hematological, internal-secretory and serophysiological functions of the entire organism (9).

As far as the circulation is concerned, it is true that upon acidification of the duodenum a slight but distinct lowering of the blood pressure occurs, accompanied by contraction of the spleen (11). During the blood pressure lowering, a transitory reduction of the depressor reflex occurs via the carotid sinus. Here also humoral factors are involved, as the blood pressure reduction occurs also in dogs with crossed circulation (10). By activation of the villikinin, the activity of the intestinal villi is greatly stimulated and their capillary network filled more (7, 17).

On the basis of these results, the question arises what changes occur in the hepatic circulation upon acidification of the duodenum. This question is of importance because in the general metabolic process the liver plays a decisive role. This is true in particular during the phase of digestion and resorption, for the initiation of which the acidification of the duodenum is important.

Method

The experiments were carried out on dogs of both sexes, weighing 10-23 kg, kept fasting for 24 hours, under chloralose anesthesia (0.1 g/kg).

The blood flow in the liver and renal cortex was measured by measuring the thermal conductivity (Hensel and Ruef, 6) and recorded continuously with a two-channel direct recorder ("Fluvograph", Hartmann & Braun A.G., Frankfurt/Main). In measuring the blood circulation in the liver, the test results of Graf. Golenhofen and Hensel (5) were assumed to be orientative. The thermal conductivity probes were produced according to the instructions of Betz, Braasch and Hensel (1). The blood circulation curves were evaluated percentually, taking the blood circulation at rest measured at the beginning of the test as 100% and the zero circulation obtained immediately after the animal was killed as 0%.

After subcostal or median laparotomy on the right, the probes were introduced into the right medial lobe of the liver or into the cortical substance of the lower pole of a kidney. At the same time a thin rubber hose was introduced through a small opening of the intestinal wall, opposite the attachment of the mesenterium, into the upper section of the duodenum, where it was secured. The wires of the probes or the rubber hose were brought out to the surface of the body and the abdominal cavity closed. The blood pressure was measured by means of a mercury manometer in the A. femoralis and recorded with a kymograph synchronized with the Fluvograph.

Hydrochloric acid at a concentration of 0.35% was given intraduodenally at body temperature in quantities of 2.5 to 3.0 ml/kg, in a total of 8 animals in 18 cases. As a control, the same quantities of physiological salt solution was injected in 9 cases, again intraduodenally.

For the statistical evaluation of the results Student's "t" test was used.

Results and Discussion

Our test results are summarized in the tables and illustrated in the figure. As can be seen from Table I, the blood circulation of the liver was considerably increased in all animals by an intraduodenal injection of hydrochloric acid. The effect varied between 5 and 45%; the average value of the experi-

ments was +21%. The phenomenon appeared in a relatively short time and had vanished after 5 to 16 minutes. Generally, greater changes also lasted longer. The half-value time of the response was on the average 3.6 minutes. From the statistical calculations the results were found to be highly significant. In agreement with our earlier observations, the arterial blood pressure showed a slight (average 13.5%) but significant ($p < 0.001$) reduction (10, 11). The organ specificity of the change in blood circulation is indicated by the observation that in all experiments the acidification of the duodenum exerted no appreciable effect on the blood circulation in the renal cortex.

Table I

Effect of intraduodenal HCl supply (0.35%; 2.5-3.0 ml/kg) on blood circulation in the liver.

Test	Initial value of blood flow in %	Maximum change Flow %	Time min.	Duration of change min.	Halfvalue time of change min.
1	102	118	1.5	5.0	3.0
	80	94	1.5	6.0	2.5
2	100	118	1.5	7.0	3.0
	100	112	2.5	7.0	3.5
3	92	120	1.0	6.0	2.5
	92	109	1.5	5.0	2.5
4	100	105	1.0	5.0	2.0
	81	90	2.0	8.5	3.0
	78	88	2.0	6.0	3.0
5	95	101	1.5	4.0	3.0
	76	82	2.5	6.0	4.0
6	100	140	3.5	6.5	4.5
	85	130	1.5	13.0	5.0
	90	120	2.5	14.0	6.0
7	91	133	1.5	15.5	5.0
	100	128	2.0	7.0	4.0
	105	129	2.0	8.5	5.5
8	90	112	1.5	5.0	3.0
SUMx	1657	2037	33.0	135.5	65.0
$\bar{x}$	92	113	1.83	7.54	3.6
p	<0.001				

In one test series physiological salt solution was injected into the duodenum instead of hydrochloric acid. The tests showed on the average a change of +7.5%. But due to the scatter of the results, this value cannot be regarded as significant ($0.20 > p > 0.10$).

Fig. 1. Effect of intraduodenal application of NaCl and HCl on the blood pressure (A. femoralis) and blood flow in the liver and kidney in dogs under chloralose anesthesia.

One experiment is illustrated by Fig. 1 (third experiment). Physiological NaCl solution given intraduodenally in a dose of 2.5 ml/kg had no effect on the blood flow in the liver or in the kidney. But if under the same conditions 0.35% HCl solution was injected into the duodenum, the blood pressure was reduced slightly but distinctly. It stayed at this reduced pressure for a shorter or longer time and gradually returned to the starting value. This is in line with our previously reported findings (10, 11). During this time, the blood circulation in the liver has increased markedly, while the renal circulation underwent no major change. As Tables I and II show, we obtained similar results in the other tests. The fact that after pretreatment of the mucous membrane of the duodenum with lidocaine (0.5-1.0%) the effect was greatly reduced suggests a nerve effect.

Table II

Effect of intraduodenal introduction of physiol. NaCl solution (2.5-3 0 ml/kg) on the blood flow in the liver.

Test	Initial value in %	Maximum variation, in %
1	100	107
2	100	110
	72	78
3	100	93

Table II continued.

4	100	100
5	100	90
6	100	135
	96	117
7	100	105
SUMx	868	935
$\bar{x}$	96.5	104
p	$0.20 > p > 0.10$	

Our research has demonstrated, therefore, that acidification of the duodenum temporarily increases the blood circulation in the liver. This occurs several times daily for a shorter or longer time, regardless of food intake. Considering that there is a close relationship between liver function and blood flow (3), the important role of this phenomenon becomes evident. Upon acidification of the duodenum - this phenomenon occurs as an initiation of the phase of digestion and resorption - the blood flow in the liver is increased always when this is especially necessary for a livelier metabolic process. It has been proven before that hepatic blood flow is increased by ingestion of food (2).

It must be discussed further what the mechanism of this phenomenon is. In the first phase of the so-called "dumping reaction" the excitation of the sympathico-adrenal system can be demonstrated by means of the enterovisceral reflex (15). It is known that acidification of the duodenum can bring about a "dumping reaction" (12) in the dog (9) as well as in man (13). The hepatic blood flow can be increased also by adrenalin (16, 4). It will be the purpose of further investigations to decide what the role of humoral factors is besides the nervous effects. Research in this direction is under way.

In this connection also the findings of Demling and Ottenjann (14) are of importance. The results of these authors, obtained by means of thermal conductivity measurement on the sigmoid mucosa in man during the "dumping reaction", permit in certain cases conclusions as to the increase of the portal circulation.

While we do not intend to go into the details of increased blood circulation of the liver, we should mention that this is of interest from the clinical standpoint. Besides the "dumping reaction", reference must be made to the different secretional activity of the stomach, as well as to the fact that in conditions after gastric operations, ulcers, enteritis and many others the morphological as well as the functional conditions of the duodenum are changed. It may be assumed that this explains why after Billroth II more late hepatic lesions have been described than after Billroth I (8). Whatever modifies the described mechanism concerns also, via the liver function, the entire body. Other effects that can be brought about on the hepatic circulation via the duodenum are being examined at present. The additional blood supply to the liver capable of being caused in the manner described must also be considered as a possible therapeutic effect.

(For Summary in English see p. 323 of original publication)

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(*) Dedicated to Prof. F. Verzar (Basel) on his 80th birthday.

Received: March 3, 1965.

Translated by Carl Demrick Associates, Inc./LH/db

Benyo-'65
Bib no. 34

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Med. Pharmacol. Expt. 317-324. 1965.

Med. Pharmacol. exp. *13:* 317–324 (1965)

Aus dem Physiol. Lab. der II. Chirurgischen Klinik und dem Forschungslaboratorium für experimentelle Medizin der Medizinischen Universität, Budapest

Die Wirkung der Duodenumsäuerung auf den Leberkreislauf*

Von I. Benyó, Fr. Füsy, P. Görgő, G. Ludány und A. Mitsányi

Durch die Untersuchungsergebnisse der letzten Jahre ist erwiesen, daß die Säuerung des Duodenums zahlreiche Funktionen des Organismus auslöst und lenkt. Diese Veränderungen beziehen sich nicht nur, wie allgemein angenommen, auf die Mikro-, Makromotilität und Sekretionstätigkeit des Magendarmtraktes, sondern sie beeinflussen auch die Resorptions- und Permeabilitätsverhältnisse des Darmes sowie die hämatologischen, innersekretorischen und sero-physiologischen Funktionen des ganzen Organismus (9).

Soweit es den Kreislauf betrifft, tritt bei Säuerung des Duodenums zwar eine geringe, aber deutliche Senkung des Blutdrucks ein, welche von einer Milzkontraktion begleitet ist (11). Während der Blutdrucksenkung tritt eine vorübergehende Verminderung des Depressorreflexes über den Carotissinus auf. Dabei spielen auch humorale Faktoren eine Rolle, da die Blutdrucksenkung auch bei Hunden mit gekreuztem Kreislauf eintritt (10). Durch Aktivierung des Villikinins wird die Tätigkeit der Darmzotten stark angeregt und ihr Kapillarnetz stärker gefüllt (7, 17).

Auf Grund dieser Ergebnisse ergibt sich die Frage, welche Veränderungen im Leberkreislauf bei Säuerung des Duodenums auftreten. Diese Frage ist von Bedeutung, da der Leber im all-

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* Herrn Prof. *F. Verzár* (Basel) zu seinem 80. Geburtstag ergebenst gewidmet.

Für geleistete technische Hilfe danken wir Herrn *G. Szilassy, L. Varga* und Frl. *M. Fekete.*

Eingegangen: 3. 3. 1965.

gemeinen Stoffwechselprozeß eine entscheidende Rolle zukommt. Dies gilt insbesondere bei der Verdauungs- und Resorptionsphase, für deren Einleitung die Säuerung des Duodenums wichtig ist.

Methodik

Die Experimente wurden an 10—23 kg schweren, 24 Stunden lang hungernden Hunden beiden Geschlechtes in Chloralose-Narkose (0,1 g/kg) durchgeführt.

Die Durchblutung der Leber und der Nierenrinde wurde nach dem Prinzip der Wärmeleitmessung (*Hensel und Ruef*, 6) gemessen und mit einem zweikanaligen Direktschreiber («*Fluvograph*», Hartmann & Braun A.G., Frankfurt Main) fortlaufend registriert. Bei der Messung der Leberdurchblutung wurden die Untersuchungsergebnisse von *Graf, Golenhofen und Hensel* (5) als richtungweisend angenommen. Die Wärmeleitsonden wurden nach den Angaben von *Betz, Brausch und Hensel* (1) angefertigt. Die Durchblutungskurven wurden prozentual ausgewertet, wobei die zu Beginn des Versuches gemessene Ruhedurchblutung als 100% und der unmittelbar nach Tötung des Tieres gewonnene Durchblutungsnullwert als 0% gesetzt wurden.

Die Sonden wurden nach rechtsseitiger subcostaler oder medianer Laparatomie in den rechten medialen Lappen der Leber bzw. in die Rindensubstanz des unteren Poles einer Niere eingeführt. Gleichzeitig wurde durch eine kleine Öffnung der Darmwand, gegenüber des Ansatzes des Mesenteriums, in den oberen Abschnitt des Duodenums ein dünner Gummischlauch eingeführt und befestigt. Die Drähte der Sonden bzw. der Gummischlauch wurden auf die Oberfläche des Körpers hinausgeführt und die Bauchhöhle verschlossen. Der Blutdruck wurde mittels eines Quecksilbermanometers in der A. femoralis gemessen und mit einem mit dem Fluvograph synchronisierten Kymograph registriert.

Es wurde Salzsäure von 0,35%iger Konzentration in 2,5—3,0 ml/kg Mengen bei Körpertemperatur intraduodenal verabreicht, insgesamt bei 8 Tieren in 18 Fällen. Als Kontrolle wurden in 9 Fällen die gleichen Mengen von physiologischer Kochsalzlösung ebenfalls intraduodenal eingespritzt.

Zur statistischen Auswertung der Ergebnisse wurde der «t»-Test nach *Student* benützt.

Ergebnisse und Besprechung

Unsere Versuchsergebnisse sind in den Tabellen zusammengefaßt bzw. in der Abbildung veranschaulicht. Wie aus der Tabelle I ersichtlich, wurde durch eine intraduodenale Salzsäureinjektion bei sämtlichen Tieren die Durchblutung der Leber bedeutend erhöht. Der Effekt schwankte zwischen 5—45%; der Durchschnittswert der Experimente betrug + 21%. Die Erscheinung trat in verhältnismäßig kurzer Zeit auf und war nach 5 bis 16 Minuten abgeklungen. Im allgemeinen dauerten größere Ver-

änderungen auch längere Zeit. Die Halbwertzeit der Antwort betrug im Durchschnitt 3,6 Minuten. Nach den statistischen Berechnungen hat sich eine starke Signifikanz der Resultate ergeben. Der arterielle Blutdruck zeigte im Einklang mit unseren früheren Beobachtungen eine geringe (im Durchschnitt 13,5%ige), aber signifikante ($p < 0{,}001$) Verminderung (10, 11). Auf die Organspezifität der Durchblutungsveränderung weist die Beobachtung hin, daß die Säuerung des Duodenums in sämtlichen Versuchen auf die Durchblutung der Nierenrinde keinen nennenswerten Einfluß ausgeübt hat.

In einer Versuchsreihe wurde anstatt Salzsäure physiologische

TABELLE I

Die Wirkung von intraduodenaler HCl-Einführung (0,25%; 2,5—3,0 ml/kg) auf die Durchblutung der Leber

Versuch	Ausgangswert der Durchströmung in %	Maximale Änderung		Dauer der Änderung in Min.	Halbwertszeit der Änderung in Min.
		Durchströmung in %	Zeit in Min.		
1.	102	118	1,5	5,0	3,0
	80	94	1,5	6,0	2,5
2.	100	118	1,5	7,0	5,0
	100	112	2,5	7,0	3,5
3.	92	120	1,0	6,0	2,5
	92	109	1,5	5,0	2,5
4.	100	105	1,0	5,0	2,0
	81	90	2,0	8,5	3,0
	78	88	2,0	6,0	3,0
5.	95	101	1,5	4,0	3,0
	76	82	2,5	6,0	4,0
6.	100	140	3,5	6,5	4,5
	85	130	1,5	13,0	5,0
	90	120	2,5	14,0	6,0
7.	91	133	1,5	15,5	5,0
	100	128	2,0	7,0	4,0
	105	129	2,0	8,5	5,5
8.	90	112	1,5	5,0	3,0
Σx	1657	2037	33,0	135,5	65,0
$\bar{x}$	92	113	1,83	7,54	3,6
p		$< 0{,}001$			

Kochsalzlösung in das Duodenum eingespritzt. Die Untersuchungen haben im Durchschnitt eine Veränderung von + 7,5% gezeigt. Infolge der Streuung der Ergebnisse kann aber dieser Wert nicht als signifikant angesehen werden ($0{,}20 > p > 0{,}10$).

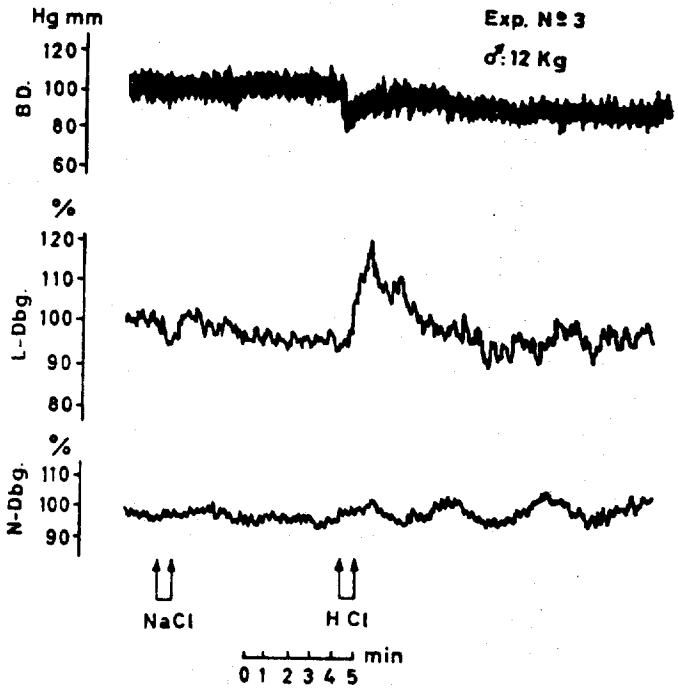

Abb. 1. Die Wirkung von intraduodenaler NaCl- und HCl-Einführung auf den Blutdruck (A. femoralis) und Durchblutung der Leber und Niere beim Hunde unter Chloralose-Narkose.

Ein Experiment wird durch die Abb. 1 veranschaulicht (3. Experiment). Intraduodenal verabreichte 2,5 ml/Kg physiologische NaCl-Lösung hat weder die Durchblutung der Leber noch der Niere beeinflußt. Wurde aber unter gleichen Umständen 0,35%ige HCl-Lösung in das Duodenum injiziert, so verminderte sich der Blutdruck zwar gering, aber deutlich. Er hielt sich kürzere oder längere Zeit bei diesem erniedrigten Druck und erreichte allmählich wieder den Ausgangswert. Dies entspricht unseren schon früher mitgeteilten Feststellungen (10, 11). Während dieser Zeit hat sich die Durchblutung der Leber ausgesprochen gesteigert; demgegenüber blieb die Nierendurchblutung ohne wesentliche

TABELLE II

Die Wirkung von intraduodenaler Einführung von physiol. NaCl-Lösung (2,5—3,0 ml/kg) auf die Durchblutung der Leber

Versuch	Ausgangswert in %	Maximale Änderung in %
1.	100	107
2.	100	110
	72	78
3.	100	93
4.	100	100
5.	100	90
6.	100	135
	96	117
7.	100	105
Σx	868	935
$\bar{x}$	96,5	104
p	$0{,}20 > p > 0{,}10$	

Veränderung. Nach den Angaben der Tabelle I und II gelangten wir in den übrigen Versuchen zu ähnlichen Ergebnissen. Es spricht für eine Nervenwirkung, daß nach vorheriger Behandlung der Schleimhaut des Duodenums mit Lidocain (0,5—1,0%) der Effekt stark abgeschwächt wurde.

Auf Grund unserer Untersuchungen wurde also erwiesen, daß die Säuerung des Duodenums die Durchblutung der Leber vorübergehend steigert. Dies erfolgt, abhängig von der Nahrungsaufnahme, mehrmals täglich für kürzere oder längere Zeit. Wird berücksichtigt, daß zwischen der Leberfunktion und der Durchblutung eine enge Beziehung besteht (3), so ist die wichtige Rolle dieses Phänomens augenscheinlich. Bei Säuerung des Duodenums — diese Erscheinung tritt als Einleitung der Verdauungs- und Resorptionsphase auf — erhöht sich die Leberdurchblutung immer dann, wenn dies wegen eines regeren Stoffwechselprozesses besonders nötig ist. Es wurde bereits früher bewiesen, daß durch die Nahrungsaufnahme die Leberdurchblutung gesteigert wird (2).

Weiter muß diskutiert werden, welchen Mechanismus diese Erscheinung besitzt. Bei der ersten Phase der sog. «Dumping-Reaktion» kann mittels des enterovisceralen Reflexes die Erregung des sympathico-adrenalen Systems nachgewiesen werden (15). Es

ist bekannt, daß durch Säuerung des Duodenums sowohl beim Hund (9) als auch beim Menschen (13) eine «Dumping-Reaktion» ausgelöst werden kann (12). Auch kann durch Adrenalin die Leberdurchblutung gesteigert werden (16, 4). Es wird die Aufgabe weiterer Untersuchungen sein, zu entscheiden, welche Rolle neben den nervösen Einwirkungen den humoralen Faktoren zukommt. In dieser Richtung sind Untersuchungen im Gange.

In diesem Zusammenhang sind auch die Befunde von *Demling und Ottenjann* (14) von Bedeutung. Die Ergebnisse dieser Autoren, welche mit Hilfe der Wärmeleitmessung an der Sigma-Schleimhaut beim Menschen während der «Dumping-Reaktion» gewonnen wurden, lassen in gewissen Fällen Schlüsse auf die Steigerung des portalen Kreislaufes zu.

Obwohl auf die Durchblutungssteigerung der Leber nicht näher eingegangen werden soll, muß erwähnt werden, daß diese vom klinischen Standpunkt aus Interesse beansprucht. Es muß hier neben der «Dumping-Reaktion» auch auf die abweichende Sekretionstätigkeit des Magens hingewiesen werden. Ebenso darauf, daß sich bei Zuständen nach Magenoperationen, bei Ulcus, Enteritis und vielen anderen die morphologischen sowie funktionellen Verhältnisse des Duodenums verändern. Es kann angenommen werden, daß mit den erwähnten Tatsachen der Umstand erklärt werden kann, daß nach *Billroth* II mehr Leberspätschäden beschrieben wurden als nach *Billroth* I (8). Jeder Umstand, der den beschriebenen Mechanismus modifiziert, betrifft auch über die Leberfunktion den ganzen Körper. Andere Effekte, welche über das Duodenum am Leberkreislauf ausgelöst werden können, werden derzeitig untersucht. Die auf die beschriebene Weise auslösbare Mehrdurchblutung der Leber muß auch als evtl. therapeutischer Effekt bedacht werden.

Zusammenfassung

An Hunden in Chloralose-Narkose wurde mittels der Methode zur Durchblutungsmessung nach *Hensel* festgestellt, daß nach intraduodenaler Salzsäureinjektion (0,35%ige HCl; 2,5—3,0 ml/kg) die Durchblutung der Leber im Durchschnitt mit 21% in signifikanter Weise gesteigert wurde. Der Effekt dauert 5—16 Minuten. Während dieser Zeit erfolgt eine geringe, aber deutliche Blutdrucksenkung. Die Nierendurchblutung wird nicht beeinflußt. Intraduodenal verabreichte physiologische NaCl-Lösung ist wirkungslos. Es wird auf den Mechanismus und auf die klinischen Beziehungen dieses Effektes hingewiesen.

Summary

Using *Hensel's* method of blood flow determination in dogs under chloralose anaesthesia it was established that after intraduodenal injection of hydrochloric acid (0.35% HCl; 2.5-3.0 ml/kg) hepatic circulation is significantly increased by an average of 21%. The effect lasted 5-16 minutes. During this time there was a small but definite lowering of blood pressure. Renal circulation was not affected. Intraduodenal administered physiological saline has no effect. The mechanism and clinical implications are indicated.

Résumé

Chez des Chiens chloralosés, il a été démontré par la méthode de mesure du débit sanguin de *Hensel*, que l'injection intraduodénale d'acide chlorhydrique (2,5 à 3,0 ml/kg de HCl à 0,35 %) augmente le débit sanguin hépatique de 21 % en moyenne. L'effet dure de 5 à 16 minutes. Pendant ce temps, on observe une hypotension faible, mais nette. La circulation sanguine dans les reins n'est pas influencée. L'injection intraduodénale de solution de NaCl physiologique est sans action. Les mécanismes et les incidences cliniques de cet effet sont discutés.

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Beznak-'42
Bib No. 38

Arch. int. Pharmacodyn., 1942, LXVII, No 2.

FROM THE INSTITUTE OF PHYSIOLOGY OF THE PÉTER PÁZMÁNY UNIVERSITY, BUDAPEST

THE RÔLE OF THE INCREASE IN THE CONCENTRATION OF CERTAIN BLOOD CONSTITUENTS IN THE PRODUCTION OF SUPRARENAL HYPERTROPHY CAUSED BY MUSCULAR EXERCISE *

BY

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(Eingegangen am 28-9-1941.)

Suprarenal hypertrophy has been observed in widely different conditions and now with the intensification of research on suprarenal cortical physiology certain interest is focused on it. Although a somewhat detailed review of its literature would not be out of place—we shall restrict ourselves in the following lines to the relation of our work on suprarenal hypertrophy observed in the albino rat during forced exercise to the theory of "alarm reaction" as advanced by SELYE and his co-workers.

Increase in the wet weight of the suprarenals in consequence of exercise appears to be a definitly established fact. First noted by HATAI (1915) it was confirmed or rediscovered by BOROVANSKY (1930), by DONALDSON and MEESER (1932). Independently from these authors it was also observed by BEZNÁK and SARKADY (1933) that several hours of daily exercise causes a 20 % suprarenal hypertrophy in young 130-140 g. albino rats of both sexes during a period of about 130 days of exercise. Using a histological reconstruction method, they concluded that the hypertrophy is a cortical one. At that time it was already known on the one hand that the absence of the vitamin B complex from the food causes suprarenal cortical hypertrophy (BEZNÁK, 1924,

(*) This paper was read to the Hungarian Physiological Society 26-28.V.1938 (*Physiol. Ber.* 108, 665, 1938). Circumstances outside the authors control prevented it's earlier publication in extenso.

VERZÁR and PÉTER, 1924) and on the other that the vitamin B requirement is directly proportional to the metabolism (COWGILL, SMITH and BEARD, 1925). Basing their assumption on these observations BEZNÁK and SARKADY (*l.c.*) suggested that the suprarenal cortical hypertrophy observed during exercise is the consequence of a relative lack of the vitamin B complex, due to the increase of metabolism consequential to the exercise. They postulated that if the suprarenal hypertrophy caused by exercise is actually the consequence of a relative lack of the B-vitamins then in the working rats it should be preventable by an increase of the B vitamins in the diet, proportional to the increased exercise. Two years later it was indeed found by BEZNÁK and PERJÉS (1935) that the increase of the wet weight of the suprarenals does not take place if the working rats receive five times as much yeast extract as do the resting ones. This experiment appeared to supply the evidence, therefore BEZNÁK and SARKADY's (*l.c.*) conclusion must be regarded as correct.

The work on this question was continued in two directions : *a*) experiments were carried out to identify the factor in the yeast extract capable of inhibiting the suprarenal hypertrophy, *b*) the other line of attack aimed at the elucidation of the sequence of physiological events which in the working rat leads to the hypertrophy of the suprarenals.

While these experiments were in progress the observation was published by SELYE (1936 and 1937) that severe injury inflicted upon the organism leads to a characteristic syndrome, " the symptome of which are independent of the nature of the damaging agent or the pharmacological type of the drug employed, and represents rather a response to damage as such." The syndrome is considered by the author " to be the expression of a general alarm of the organism when suddenly confronted with a critical situation," and is regarded " to represent a generalized effort of the organism to adopt itself to new conditions." The names of " general alarm reaction " and " general adaptation syndrome " were suggested to denote this phenomenon.

One of the symptomes of SELYE's " alarm reaction " is an increase of the wet weight of the suprarenals, moreover physical exercise is one of the non-specific stimuli, which when allowed to act in sublethal strength is capable of producing the alarm-reaction. Since our present work deals also with the phenomenon of suprarenal hypertrophy in exercise, the differences between SELYE's and our own work must be made clear. In the course of our experiments we have not been dealing with the effects of so strong an exercise as is capable of causing,

non-specifically by its strength, severe symptoms of exhaustion i.e. provoking the alarm-reaction. Increase in the weight of the suprarenals appears to be an early effect of the exercise, as proved by the following observations : DONALDSON and MEESER (*l.c.*) observed it without finding any sign of alarm in several generations of rats doing voluntary exercise. It is clear that rats taking voluntary exercise will not carry this to such an excess as to cause an "alarm reaction", yet the suprarenal hypertrophy was present. Indeed DONALSDON and MEESER (*l.c.*) emphasize in the course of their study of the changes of the weight of 15 organs after voluntary exercise that " the heart and the suprarenals are especially highly responsive to exercise." A cumulative consideration of the organ changes, body weight and body growth indicate rather a favourable effect of exercise than an " alarm reaction." BEZNÁK and SARKADY (*l.c.*) studying forced exercise also without finding signs of an alarm, observed the growth curve of the working rats—though somewhat depressed—almost normal, yet they found suprarenal hypertrophy. D. ANDERSEN (1936) also observed a suprarenal hypertrophy after a few hours of exercise. Also in the course of our present work we found that without any sign of exhaustion or alarm, suprarenal hypertrophy manifests itself. Suprarenal hypertrophy—in the excercising rat—appears therefore to be an early change and our aim was to discover experimentally the sequence of physiological events which leads to the early increase of weight of the suprarenals.

The working hypothesis directing our experiments, was as follows : the stimulus provoking the hypertrophy may reach the suprarenal cells either through their nerve supply or through the blood stream. In this latter case the stimulus causing hypertrophy is the alteration in the chemical structure of the blood, from which the working muscle takes out constituents and into which it adds break-down products. HASCH (KORÉNYI) and HAJDU (1939) dealt with the possibility of cholesterol added to the blood by the contracting muscle as a possible molecular stimulus of the hypertrophy. They found that the sera of both normal and excercising dogs when injected into rats cause a hypertrophy. No greater difference was found between the hypertrophies caused by the resting and working dogs' serum respectively. The former caused a 30 %, the latter a 38 % hypertrophy. HASCH and HAJDU presented very good evidence proving that the substance in the injected blood serum causing the hypertrophy of the suprarenals is cholesterol. The cause of the fact that the blood serum of the resting and exercising dogs provoke about [illegible]

working period (when the blood was taken from the dogs) the blood cholesterol content was about the same in the inactive as well as in the exercising dogs. In the rat itself whose suprarenal enlarges during exercise a hypercholesterolaemia develops at the beginning of the exercise. In spite of the facts that cholesterol when injected causes hypertrophy and that there is a hypercholesterolaemia at the beginning of the exercise HASCH and HAJDU (*l.c.*) do not consider this initial hypercholesterolaemia to be the cause of the enlargement of the suprarenals because during exercise the hypercholesterolaemia does not last long enough and is not high enough.

While HASCH and HAJDU (*l.c.*) found that the hypertrophy of the suprarenals in the *rat* caused by the serum of the exercising *dog* is only 27 % greater than that caused by the serum of the resting dog, RIML (1937 and 1938) found that the dialyzates of the sera of patients with tetanus convulsions or that of runners, causes, when injected into guinea-pigs an enlargement which is 40 % greater than the weight of the suprarenal of such guinea-pigs which have been injected with the serum dialyzate of inactive men. Since cholesterol does not pass celophane RIML's conclusion is in harmony with that of HASCH and HAJDU (*l.c.*) who also suggested that the chemical mediator must be an other substance but cholesterol.

In the present paper we are reporting new experiments carried out in order to answer the following questions : *Do any of the organic compounds (other than cholesterol) or inorganic ions, the concentration of which is known to increase during exercise and are dialyzable, cause a hypertrophy of the suprarenals? Which of them is the most powerful in causing hypertrophy? How does the concentration of the most effective agents change during exercise? Is it possible to provoke suprarenal hypertrophy by imitating the concentration changes of these compounds as they take place during exercise by repeated injections of those compounds?*

Reference should be made also at this point to SELYE's observations (*l.c.*). He found that different kinds of chemicals when administered in sublethal doses, provoke the " syndrome of alarm " irrespective of the specificity of the substance applied. We, on the other hand, wanted to find out if there is a substance (or if there are several substances) produced in the body under physiological conditions, specifically responsible for the increase in the weight of the suprarenals *under physiological conditions* in non toxic doses, without producing in physiological doses the symptoms of the alarm reaction.

METHODS

The experiments were carried out from September 1937 untill the middle of May 1938. Male albino rats were used. For each type of experiment rats of closely similar body weight (180 g)were selected, the individual weight never deviating from the average by more than ± 20 %. The rats lived in large cages in groups of not more than 12 and received a diet of mixed cereals. This was balanced in regard to protein-, fat-, and carbohydrate-content and was complemented with salts and vitamins. For details an earlier paper by two of us should be consulted (Z. HASCH-KORÉNYI and I. HAJDU, 1939). The injections were mostly given intraperitoneally and in some cases subcutaneously. Each substance was dissolved in distilled water and the amount used was given in 1 cc. volume. For any further details see the description of the experimental results.

At the end of the experimental period the rats were stunned but not bled out, the suprarenals carefully removed, cleared of fat, the left and right one being weighed separately on the torsion balance within 2 minutes after stunning. Separate experiments proved that if the suprarenals were allowed to dry on the torsion balance, within 5 minutes no measurable loss of weight occurred.

In a series of experiments the K-ion, the cholesterol, and the total organic acid contents of the rat's blood serum was determined. The potassium determination was carried out using the method of SHOHL and BENETT (1928) the organic acids with that of VAN SLYKE as modified by CSAPÓ and KERPEL-FRONIUS (1933) and the cholesterol with that of MEYERS-WARDELL (1918).

RESULTS

First of all, we present an example of the exercise causing suprarenal hypertrophy. The exercise consisted in running a tread-mill having a velocity of 1 km/hour. The rats were divided into 2 groups. Those in the 1st covered 37.5 kms. during 13 days, those in the second 70 kms. during 21 days. The rats were gradually trained to their maximum capacity of work. During the first day they ran only 1, in the last days 5 hours a day. They were fed on the usual ration (HASCH and HAJDU, 1939) and were supplied with tap water. The animals were killed 24 hours after the last day's run and, the wet suprarenals having been weighed, were compared with those of the normal resting rats of the same weight, living in the same lab. side by side with the [illegible]

rats (TABLE I). During the three weeks of experiments mortality did not occur. The running animals showed no signs of a "generalized alarm reaction."

TABLE I

The effect of exercise on the wet weight of the suprarenals in the rat.

A	Type of group	No. of rats	Average of the two suprarenals		Hypertrophy in	
			mg.	$\mu\pm$	mg.	%
	1	2	3	4	5	6
B	Control	11	31.0	4.2	—	—
C	37.5 kms run in 13 days	19	40.6	9.4	9.6	31
D	70 kms run in 21 days	11	47.1	6.3	16.1	52

In the first series of our injection experiments, the blood serum level of urea, of creatinine, of acetic acid, of HCl, of KCl, of NaCl, of NaH_2PO_4 had been maintained by repeated intraperitoneal injections at an elevated level for 4 days. The ability of these substances to cause suprarenal hypertrophy had first been subject to a preliminary test in the following way :

A single dose of each of the above substances was given to rats. In the cases of KCl, NaCl, NaH_2PO_4, urea and creatinine the doses given were 3-, 6-, 12-, 25-, and 50-times as much as is found of each of them in the blood or serum of a single rat. In the case of acids we proceeded as follows : The total H-ion contents of organic acid origin was determined in the rats' serum, then as much acetic or hydrochloric acid was given, as contained 0.75-, 1.6-, 3.1-times as much H-ions, as is normally present in the organic acids of the serum of a single rat. All injections were given in 1 cc. of distilled water intraperitoneally. For each substance and each dose a different rat was used. They were killed 12 hours after the injection. On weighing the suprarenals, it became evident that whereas NaCl, and NaH_2PO_4 do not cause a hypertrophy in any of the cases, acetic acid, HCl, KCl, urea all cause hypertrophy, even in the smallest dose (i.e. 3 times as much as is present in the blood); creatinine was also effective, but only if at least 25 times the normal blood serum quantity was given.

After this preliminary test, the minimum hypertrophy producing dose of each substance, generally 3 times as much as is present in the rat's blood, was given to another series of animals. From acetic and

hydrochloric acids—however—0.75 and 1.6-times the normal amounts were also administered. The injections were made into groups of rats, each group consisting of 5-8 rats according to the following schedule : at 0.12, 24 hours, then followed a 36 hours rest, after this the second series of injections at 0.12, and 24 hours. 12 hours after the last injections the animals were killed. During the experimental period the rats consumed their normal food and drink. In our experiments we studied the effect of that minimum amount of a substance which is just capable of producing hypertrophy. Such small doses although provoking suprarenal hypertrophy do not cause any visible change in the appearance of the animals. Large doses of some of these substances, on the other hand, cause many different symptoms and a sufficiently massive dose may even be lethal. Table II shows the weights of the suprarenals after such treatments.

From the results presented in Table II it can be safely concluded

TABLE II

The effect of the injection of different substances on the wet weight of the suprarenals in the rat.

A	Type of group	No. of rats	Single dose mg.	Proportion of the single dose to its amount present in blood	Average of the two suprarenals mg.	$\mu \pm$	Hypertrophy in mg.	%
	1	2	3	4	5	6	7	8
B	Control	8	—	—	23.5	2.7	—	—
C	Acetic acid	6	5	0.75	29.0	3.4	5.5	23.4
D	Acetic acid	6	10	1.60	33.4	5.9	9.9	42.1
E	Acetic acid	6	20	3.10	39.6	5.6	16.1	68.5
F	Hydrochloric acid	6	3	0.75	39.0	6.8	15.5	65.9
G	Hydrochloric acid	6	6	1.60	39.3	5.8	15.8	67.2
H	Potassium chloride	6	10	3.00	29.5	4.8	6.0	25.5
I	Urea	5	6	3.00	27.9	2.5	4.4	18.7

that each of the substances tried, is capable of causing hypertrophy of the suprarenals, if by intraperitoneal injections their concentration is kept high enough in the blood for a sufficiently long time. It is

also evident that the ability to cause suprarenal hypertrophy is much the greatest in the case of the two acids, hydrochloric and acetic. KCl and urea come next. Further below we shall present more evidence that in the case of acids it is the H-ion and not the anion part of the molecule which is responsible for the production of the hypertrophy.

Our next problem was whether the rise of the concentration of these several substances during exercise is high and long lasting enough to account for the hypertrophy observed during exercise. In the course of this work the concentration changes of the total organic acids and of the K^+ have been determined during exercise as follows. [The cholesterol changes under similar conditions had already been determined by HASCH and HAJDU (*l.c.*)]. Groups of rats, all of about the same body weight were formed. The different groups ran a tread-mill for different lengths of time as indicated in Figure 1. In the case of the estimation of the total organic acids each group consisted of 5 rats, in that of the K^+ estimations of 9-11 rats. At the end of the respective running periods, the animals were bled out through the carotids and the determinations carried out immediately after the formation of serum was finished. For the organic acid determination the blood of the 5 rats was united.

As it is clearly seen in Figure 1, the K-contents of the blood serum

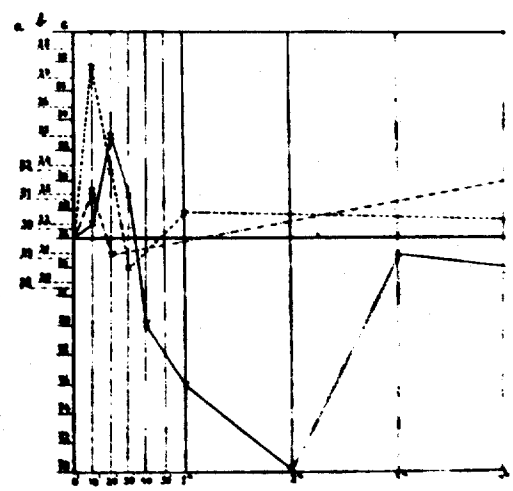

FIG. 1

The changes of the H- and K-ion content of the blood serum and that of the blood cholesterol at different durations of the exercise in rats. *x* axis: duration of the exercise. *y* axis: concentration of these substances in the blood. *a* = —.—.—.— = K in mg. %. *b* = — — — — = % organic acid in cc. $\frac{n \text{ to } H^+}{100 \text{ cc.}}$ *c* = cholesterol mg. % = ———

did not show any significant change. The total organic acid content, on the other hand, showed a quick rise at the beginning of the work, reaching it's maximum in the 10th minute. This is a transitory rise, in the 30th minute the total organic acid contents is slightly below the normal, but after this time stabilizes itself on a slightly elevated level throughout the hours of a work period. The organic acid contents of the blood therefore behave with one exception similar to the cholesterol contents. It is interesting to compare in passing the concentration-time curve of lactic acid in the *man* [HILL, LONG and LUPTON (1924)] and in the *dog* (CAMPOS, CANNON, LUNDIN and WALKER (1928)] with the concentration-time curve of organic acids found by us in the *rat*. The lactic acid in man like the total organic acid in the rat reaches its maximum round about the 10th minute and at about the 30th minute it already approaches closely the pre-working level in spite of continued exercise. In the dog it is still high at the 30th minute, though it is probable that it was even higher at the 10th minute of the exercise, but determinations were not carried out at that point of the time curve. In the case of the cholesterol (HASCH and HAJDU, *l.c.*), its concentration after the initial rise sinks much below the normal, and remains so throughout the work. The explanation of the behaviour of the time-concentration curve of organic acids during exercise is probably the fact, that the initial increase of the concentration of these substances sets compensatory mechanisms into action which keep the blood-level of these substances near to normal.

Thus the periods during which the concentration of the substances capable of producing hypertrophy is elevated and are so different in our injection experiments and during exercise, that the types of injection experiments reported so far do not prove conclusively that the hypertrophy observed during exercise is due to that initial rise of the concentration of the hypertrophogen substances which take place at the onset of the work. In the 3rd series of injection experiments, we tried therefore to imitate the natural circumstances during exercise. For 13 days (that was the exercising period of the rats in group 1) we gave rats daily a single injection of organic acids, of KCl and of cholesterol respectively. Each group consisted of 10 rats. Three organic acids have been used, acetic, as a mono-, succinic as a di-, and citric as a tribasic acid. The following amounts were given in 2 ccs. of water subcutaneously: Acetic acid 5 mg., succinic acid 5.8 mg., and citric acid 16 mg. per animal. These are about equimolecular with the minimum amount of acetic acid that was capable of causing hypertrophy [illegible] As it

may be seen from Table III, all of them were capable of producing hypertrophy and the hypertrophy was proportional to the number of COOH groups present in the acids administered, and the structure —at any rate of these acids—of the molecule made no difference. It may also be seen from Table III, that these minimum amounts of

TABLE III

The effect of daily injections of different substances on the wet weight of the suprarenals in the rat.

A	Type of group	Control rats				Injected rats					Hypertrophy in	
		No. of cases	Body weight	Average of the two suprarenals		No. of cases	Body weight	Quantity injected daily	Average of the two suprarenals			
			g.	mg.	μ±		g.	mg.	mg.	μ±	mg.	%
	1	2	3	4	5	6	7	8	9	10	11	12
B	Acetic acid	11	200	32.0	2.7	11	200	5.0	34.0	2.6	2.0	6
C	Succinic acid	11	200	32.0	2.7	10	200	9.8	39.4	4.8	7.4	23
D	Citric acid	11	200	32.0	2.7	12	200	16.0	40.6	5.9	8.6	27
E	Cholesterol	12	230	36.0	2.8	10	230	0.5	36.0	4.0	0.0	0
F	Potassium	11	145	31.1	4.2	10	145	10.0	31.7	7.5	0.6	2

KCl, and cholesterol when given once a day for 13 days, hardly cause hypertrophy.

In a separate series of experiments we determined the cholesterol- and K-contents of the serum 30 minutes after the rats had received subcutaneously 10 mg. KCl, and 0.5 mg. cholesterol respectively. As it is evident from Table IV, these single doses only cause a slight and varying elevation, which corresponds roughly with that seen at the beginning of the work.

Similar determinations of the total organic acid contents of the blood after a single injection of acetic, succinic or citric acids have not been carried out, but it may be reasonably supposed that a similar small rise takes place.

TABLE IV

The effect of the injection of a single dose of KCl and of cholesterol respectively on the K^+ and cholesterol contents of the blood serum in the rat.

N°	Potassium		Cholesterol	
	normal mg. %	injected mg. %	normal mg. %	injected mg. %
	1	2	3	4
1	26.8	34.7	87	93
2	29.2	40.0	85	97
3	33.2	27.9	90	89
4	25.4	36.1	82	91
5	28.3		97	
6	30.7		78	
7			80	

DISCUSSION

Upon our various questions the experiments (see p. 5) gave the following answers. Most of the dialyzable organic and inorganic compounds, whose concentration increases during the exercise are capable of causing suprarenal hypertrophy if their concentration in the blood is kept high enough for a long enough period by repeated injections. These substances referred to a shypertrophogens and tried by us are as follows: acids, (organic or inorganic) KCl and urea. On this list the acids have been found the most effective agents.

The type of the concentration-changes of these physiological hypertrophogen substances during exercise was found to be essentially the same, independent of the substance. A sharp rise is seen during the first 10-20 minutes of the exercise, this is followed by a return to the resting level, or even below that level in the case of cholesterol. The maximum value is not very high, the increase being in the case of total organic acids about 20 %, in the case of cholesterol about 10 % of the resting value.

Regarding our main problem whether the suprarenal hypertrophy of the exercise is caused by the increase of the concentration of these substances in the blood, very little conclusion can be drawn from these

two experimental results alone. Obviously the two facts, one, that certain substances administered in large doses and for a long time are capable of causing hypertrophy, and the other, that the concentration of the same substances is increased to a small extent and for a short time in exercise do not warrant the conclusion that the hypertrophy of the suprarenals in exercise is caused by the small concentration increases of these substances. Such a conclusion may only be justified if the same substances are capable of causing a similar hypertrophy in equally small doses and acting for equal periods as is seen during exercise. As a matter of fact—as described in this paper we actually found that suprarenal hypertrophy *is* produced when organic acids are given once a day for a period as long as was that of the exercise and in doses as small as would raise the contents of organic acids of the blood to about the same level as is raised by the exercise. It was also observed that when KCl or cholesterol are given under similar experimental conditions hardly any hypertrophy is seen.

The conclusion appears to be warranted from these, that in the chain of physiological events which leads in exercise to the suprarenal hypertrophy the increase of the organic acid content of the blood is a link.

In discussing the capacity of acids to cause hypertrophy reference must be made to the work of RIML (1937) and (1938). As already mentioned he found that dialyzates of sera obtained from both patients with tetanic convulsions and from runners after a long run are capable of causing suprarenal hypertrophies in the guinea-pig. RIML believes that the substance in the dialyzate of the exercising organism is a specific toxic substance. The substance is believed to be an intermediary metabolite of the muscle-cells, and suggested that the function of the suprarenal cortex is the detoxication of this metabolite. Since RIML (1936) also found that the sera and dialyzate of sera of epinephrectomized rabbits when injected into guinea-pigs, besides killing the animal also causes suprarenal hypertrophy, he believes that the toxin of the epinephrectomy and the active principle of the sera of the exercising organism are identical with each other and with the toxin believed to be found by ABELOUS (1895) in the serum of epinephrectomized rabbits. Regarding the nature of the substance in the dialyzate responsible for the hypertrophy, attention must be called to the circumstance that our observations prove the sensibility of the suprarenals to acids to be fairly great, therefore it is likely that the active substances of the serum dialyzates are organic acids, liberated by the muscles. Taking but one of these, the lactic acid, its concentration raises in the blood after running, from the resting 5.6 mg. % to

150 mg. % (LAUG, 1934). The dialyzate of each cc. of the serum of the runner would contain at least 0.5 mgs. of lactic acid. RIML injected into his guinea-pigs the dialysate of 25 ccs. of serum which probably contained more than 10 mgs. of lactic acid alone, also probably some other acids as well. We have shown that 5 mgs. of acetic acids are already sufficient to cause suprarenal hypertrophy. The assumption appears to us justified that part if not all of the hypertrophy observed by RIML was caused by the organic acids present in the dialyzate of the serum of runners.

It remains to be demonstrated whether these organic acids, which are known to be the metabolites of muscular contraction are alone responsible for all the symptoms observed by RIML or besides them there is some specific toxin co-acting in the epinephrectomized animal. At the present state of experimental knowledge there is certainly no neccessity to suppose the existence of such specific toxins.

Finally the work of GY. I. FAZEKAS must be mentioned. This author observed in 1935 in chronic, lethal ammonia poisoning of the rabbit amongst a number of other changes in the composition of the blood also acidaemia. In 1939 he discovered that ammonia poisoning is accompanied by suprarenal hypertrophy. Since in a number of other suprarenal hypertrophies there is a certain acidaemia present, he advances the striking hypothesis that "Da durch die längere Verabreichung der Extrakte endokriner Drüsen (Thyroxin), Hypophysenvorderlappenextrakt, sowie anderer giftiger Stoffe ebenso wie durch die NH_4OH-Vergiftung Azidose und Lipämie oder Hypercholesterinämie hervorgerufen werden kann, ist die Hypertrophie der Nebennierenrinde nicht als Folge einer spezifischen Wirkung der Hormone oder der Gifte, sondern als Folge der auf der Intoxikation beruhenden Azidose, Lipämie bzw. Hypercholesterinämie aufzufassen. Dies besagt, dass bisher keine genügenden Anhaltspunkte zur Annahme des Vorhandenseins eines spezifischen adrenotropen Hormons vorliegen." In this connection we have to state that we do not regard FAZEKAS's assumption as experimentally proved, though on the other hand our experiments lend support to the supposition that in the suprarenal hypertrophy during exercise the organic acids play some rôle.

We would like to point out that we do not consider our experiments giving any information about the mechanism by which the increased blood organic acid content causes the suprarenal hypertrophy. This may be a direct action on the cells of the suprarenals or an indirect one, through some other organ, such as the nervous system, or the pituitary [illegible]

SUMMARY

1. When acetic, succinic, citric, hydrochloric acids, KCl, urea or creatinine respectively, are injected into rats, a hypertrophy of the suprarenals takes place. The hypertrophy may be produced by keeping the concentration of any of these substances at a continuously elevated level for several hours. The H-ions were found to be among the enumerated substances the most effective agents in producing suprarenal hypertrophy.

The amounts injected were small. No "alarm reaction" was seen in any of the experiments. Suprarenals of the rat appear to be very sensitive to the accumulation of acid metabolites in the blood and react with enlargement while the general appearance and behaviour of such animals does not differ from the untreated normal controls.

2. Determinations of the total organic acids of exercising rats, showed a 30 % elevation in the 10th minute of the exercise. The level of the blood organic acids is already returned to the normal value in the 30th minute of the exercise after which it remains for the coming hours of the working period at a slightly elevated level for several hours. It is uncertain wether the K-ion contents of the serum show in the rat any definite change during exercise.

3. When such small amounts of an organic acid are given to resting rats once a day,—as will probably raise the organic acid content of the serum to such an extent as it is raised at the beginning of an exercise in rats—for as long a period, as was the period of exercise, the suprarenals enlarge in the resting rats to about the same degree as they do in the exercising rats.

4. It is suggested that the physiological mechanism of the suprarenal hypertrophy caused by exercise is the following. During work organic acids enter into the blood from working cells, chiefly from the exercising muscles. The elevation of the concentration of the H-ions stimulates the suprarenals to enlargement. Besides the H-ions, cholesterol, K^+, urea may have a similar though much smaller effect. It remains undecided whether these substances act on the suprarenals directly or through some indirect mechanism. The participation of the medulla and cortex in the hypertrophy remains also to be investigated.

The authors have pleasure in thanking Professor A. B. L. BEZNÁK for his constant interest and suggestions in the course of this work.

One of us (ZOLTÁN KORÉNYI) expresses his gratitude to His Highness Prince PAUL [illegible] for a contribution to the expenses of this research.

[illegible] greater part of them have been defrayed from a grant to Professor A. B. L. BEZNÁK

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HYDROCHLORIC ACID INDUCED HYPOGLYCEMIA IN DIABETES MELLITUS [L'azione ipoglicemizzante dell'acido cloridrico nel diabete mellito]

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from

Minerva Medica 245-53

Translation from Italian

HYDROCHLORIC ACID INDUCED HYPOGLYCEMIA IN DIABETES MELLITUS

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The relationships between the various functional activities of the acinar tissue and the islet tissue in the pancreas have always been the basic element in the study of the pathogenesis of diabetes mellitus. Actually, since it was established that the disease occurs subsequent to alterations of the various pancreatic cytological elements and since the formulation of the hypothesis that human diabetes basically represents an altered islet tissue, the great number of studies carried out along this line have led to the discovery of insulin and therefore proved that the nature of diabetes is hormonal rather than neurogenic or autotoxic. However, questions have been raised as to whether the Langherans islets are independent formations of the pancreatic acini or whether they may be able to develop from the acini of the fully grown individual, and also whether acinar metaplasia is possible. The latter hypothesis has been vigorously attacked by Italian researchers, with the exception of Laguesse who formulated the theory claiming that an equilibrium ("balancement") exists between the various pancreatic cells, to the effect that a continuous morphological (and, therefore functional)reversibility exists between the endocrine and exocrine tissues. The recent comparative anatomical study performed by Bierry and Kollmann agrees with the theory of Laguesse regarding the possible formation of islets from the pancreatic acini.

Although there is still some controversy regarding the continuity of the islet tissue, it is currently established that the islet cells and the cells of the epithelium in the primary pancreatic tubules have a common

origin. This particular finding, independent of the identification of insulin, eliminated some of the rigid thinking characterizing the dual theory, especially following the acquisition of more information in this field.

As an example, Epstein, Rosenthal, Maechling and del De Bek, Grot and Monteleone, and, earlier, Banting and Best, have shown that insulin is inactivated by trypsin, both in vivo and in vitro, and also that insulin itself has secretion stimulating activity. Together with other investigators, Deusch and Drost also were able to induce a significant increase of external pancreatic secretion, both in man and in experimental animals (dogs), by means of insulin injection. Furthermore, Martino isolated a substance with hypoglycemia-inducing activity from the external pancreatic secretion. This particular finding is highly significant since it demonstrates the effect of the acinar tissue on the mechanism regulating blood sugar. The activity of this hypoglycemia-inducing product is also manifested in animals. Thus, the feeding of proteins to dogs fasting for several days was found to induce pancreatic secretion. Ciaccio and Racchiusa observed the occurrence of this phenomenon in dogs fasting for 24-48 hours. They were able to obtain the same response with oral administration of hydrochloric acid and also by means of a *psychic stimulus*.

Following the identification of the external pancreatic secretion capable of affecting glycemia, the researchers attempted to determine whether the activators of external pancreatic secretion (secretin and HCl) might have any effect on the endocrine secretion of the gland.

Following the identification of secretin by Bayliss and Starling in 1902, attempts were made to determine whether the intravenous and subcutaneous administration of the new product has any effect on glycemia.

Pariset, Mellamby and Delezenne failed to demonstrate any alterations in the blood sugar levels. Abelous and Soula, Lambert and Hermann reported transient hyperglycemia followed by moderate hypoglycemia. On the other hand, Troteano, Oehme and Wimmers, Penau and Simonnet, Novao Santos, Fieschi, Takacs, reported reduction of blood sugar in dogs, rabbits, as well as in man. The discrepancy seems correlated in part to the degree of secretin purification.

As a result of many studies, Zunz and La Barre arrived at the conclusion that the results obtained by Pariset were not valid. The same investigators, however, were able to confirm the validity of the results obtained by Denjab and Mellamby who reported the appearance of pancreatic secretion subsequent to the injection of purified secretin in the femoral or jugular vein. It must be kept in mind, however, that the intestinal extract is not always free of vasodepressants, especially histamine which, as is well known, produces considerable hyperglycemia and subsequent rapid return to normal blood sugar levels (as demonstrated by Chambers and Thompson, Monteleone, Carusi, and many other investigators). Furthermore, La Barre obtained a sudden increase of adrenalin levels and subsequent hyperclycemia in the adrenal venous blood following the administration of intravenous histamine in dogs with adreno-jugular anastomosis.

Actually, Parson found about 2% histamine in non-purified secretin. This particular finding induced certain researchers, (Guggenheim as an example) to identify secretin with histamine. Furthermore, according to Popielski and Takacs, histamine alone is responsible for the secretion stimulating activity manifested by intestinal extracts. However, subsequent studies by Bayliss and Starling, Dixon and Hamill, Divry, Stepp, Luckhardt, Barlow, Weaver and Koch, etc., demonstrated that secretin and histamine are

two completely different agents. Finally, Zunz and La Barre used a variety of secretin preparations obtained according to the method described by Still, Mellamby and Underhill (secretins free of hypotensive activity) and were able to demonstrate that all such substances exhibit high secretion stimulating activity together with hypoglycemia-inducing properties. Since all of these investigators also obtained reduction of blood sugar by injecting secretin in experimental animals previously subjected to acid trypsin treatment, the conclusion was drawn that the blood sugar reducing activity of the compound is not dependent on the presence of insulin (a claim made by Mellamby).

The fact is well known that hydrochloric acid present in the juice arriving in the duodenum is the main factor in the stimulation of pancreatic secretion. There was strong controversy as to the action mechanism of HCl, some claiming that it acts via the reflex nervous system while others supported the hypothesis of a humoral pathway.

It has been established that sympathetic and vagal stimulation induces the appearance of pancreatic secretion. Recent experiments carried out by Zunz, La Barre and Destree failed to produce a definite confirmation in this respect. However, it was established that pancreatic secretion appears subsequent to the introduction of HCl in the duodenum, even following the sectioning of the two vagal and the two sympathetic nerves, or after separation of the spinal cord, and also following the removal of the solar plexus and of the upper mesenteric plexus, as demonstrated by Werteimer and Lepage between 1899 and 1901, and confirmed recently by Czarnecki. At the present time, the classical experimental results obtained first by Bayliss and Starling and confirmed subsequently by many investigators, support the humoral theory regarding the secretory activity of HCl. As mentioned

earlier, hydrochloric acid yields secretin which, in turn, stimulates a high secretory activity in the pancreas, even when the latter is fully severed from any nervous connection.

Based on this information and definitely inspired by Gley (who, however, supported the existence of intimate functional relationships between the acinar tissue and the islet tissue), Freud and Saadi-Nazim attempted to determine the effect of exocrine secretion on endocrine secretion by using artificially prepared substances instead of extracts of intestinal mucosa qualified as secretin. Accordingly, they stimulated pancreatic secretion under conditions quite similar to physiological circumstances by introducing a solution of hydrochloric acid in the duodenum. These experiments were carried out in dogs and revealed a reduction of blood sugar levels generally starting at about 15-30 minutes following the introduction of HCl and lasting 1-2 hours thereafter. Moreover, the blood sugar changes were accompanied by increased pancreatic secretion.

The same experiment was carried out by Coelho and Oliveira in men free of gastrointestinal, pancreatic and liver disorders. They introduced 30 cc of a N/10 HCl solution by means of an Einhern catheter, took repeated samples of pancreatic juice and determined the trypsin and amylase activity. At the same time, they carried out determinations of sugar levels in the blood.

14 of the 20 individuals investigated displayed a decline of blood sugar at 0.5 hours following the introduction of HCl. In two other individuals, blood sugar levels increased slightly during the first 15 minutes after treatment. The increase of exocrine pancreatic secretion was ascertained by measuring enzymatic activity. The findings induced the investigators to draw the conclusion that the stimulation of external

pancreatic secretion is accompanied by an increase of internal secretion and, therefore, a condition of hyperinsulinemia which is manifested through a decline in the level of blood sugar. It must be mentioned that this physiological mechanism of blood insulin levels during external pancreatic secretion was already investigated by Gley and Hazard. In a dog whose jugular artery was anastomized with the splenic vein of another dog injected with a solution of HCl in the duodenum, these investigators determined a reduction of blood sugar levels amounting to 20% of the initial value.

Finally, highly interesting results were obtained by Zunz and La Barre with their very recent experiments (November 1930). Administration of HCl in normal dogs with chloralose induced anesthesia and in adrenalectomized dogs with chloralose induced anesthesia resulted in various degrees of blood sugar reduction in the latter, while in the former animals remained unaffected. In a second series of experiments, Zunz and La Barre performed pancreatic-jugular anastomosis in two adrenalectomized and chloralosed dogs. Subsequent administration of HCl to the donor animal resulted in rather moderate pancreatic secretion and a significant decline of blood sugar in the latter, together with a reduction of blood sugar levels which reached highly significant levels in the receiving animal even when the latter was vagotomized.

The studies of Zunz and La Barre unveiled definite proof about the functional relationship between the exocrine and endocrine pancreatic tissues, and also underlined the major role played by hydrochloric acid, one of the components of the gastric juice, in the regulation of blood sugar. Nevertheless, as physiologists, these researchers were only able to perform their studies on animals. Accordingly, I thought that it would

be highly desirable to determine whether patients with diabetes mellitus and free of digestive disorders exhibit blood sugar changes subsequent to the administration of oral hydrochloric acid.

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The study was carried out in eight patients with various degrees of diabetes mellitus. The activity of hydrochloric acid was determined upon fasting and following food intake. Non-diabetic individuals were also tested in the same manner, for control purposes. Blood sugar levels were determined according to the Bang-Cruto method.

Results Obtained in Diabetic Patients

CASE 1: R. Maria, 59 year old female; a widow. Diabetes mellitus of average severity in a patient with arteriosclerotic obesity.

For the past 4 months, the patient had displayed polydipsia, polyuria, and pollakiuria; polyphagia was initially absent. Progressive asthenia and rapid body weight loss (within a 5 month period the patient lost 10 kg). The patient was hospitalized following the appearance of glycosuria.

Examination: Obese patient. Dental alveolar pyorrhea. Visceral examination: negative; slight signs of arthritis in the left knee and left tibio-tarsal joint. Pmx 150, Pmn 80 (RR.). Urine, 2500 ml; Ps. 1033; Glucose, 20%; traces of acetone; no diacetic acid.

Glucose Tolerance Test
(50 g glucose in 250 ml of water)

	Glycemia %	Glycosuria %	Amount of urine	Total amount of glucose, in g
Fasting	2,50	0,70	185	0,13
After 0.5 hour	2,91	1,44	90	0,13
1 hour	3,36	0,61	220	0,13
2	2,90	0,63	340	0,21
3	2,88	1,08	275	0,30
4	—	0,68	240	0,16
The following morning	—	0,23	1350	0,31
			2700	1,37

The same test following the administration of 100 drops of HCl officinalis

(50 g glucose in 250 ml of water)

	Glycemia %	Glycosuria %	Amount of urine	Total amount of glucose, in g
Fasting	2,27	1,03	150	0,15
After 0.5 hour	3,23	5,60	60	0,33
1	3,35	0,50	200	0,10
2	2,78	0,50	200	0,10
3	2,22	1,11	180	0,20
4	—	2,—	75	0,15
The following morning	2,40	0,23	1500	0,34
			2365	1,37

Fasting Blood Glucose Test

Without HCl	Glycemia %		Previous administration of 50 drops of HCl officinalis	Glycemia %	
Fasting	2,61	2,53	Fasting	2,68	2,25
After 0.5 hour	2,61	2,57	After 0.5 hour	2,53	2,15
1	2,61	2,58	1	2,15	2,14
2	2,67	2,57	2	2,20	1,82
3	2,57	2,53	3	2,09	1,72
4	—	2,50	4	—	1,50

Fasting blood glucose was also determined after the patient was subjected to insulin treatment. The administration of insulin was interrupted for 24 hours before each test.

Fasting Blood Glucose Test

Without HCl	Glycemia %	Previous administration of 50 ml of a 1% HCl solution	Glycemia %
Fasting	1,90	Fasting	1,77
After 0.5 hour	1,88	After 0.5 hour	1,67
1	1,88	1	1,60
2	1,80	2	1,43
3	1,76	3	1,47

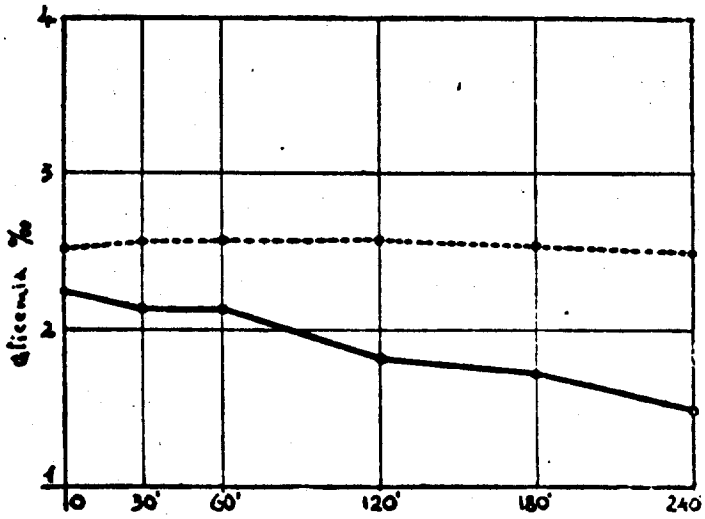

Fig. 1.

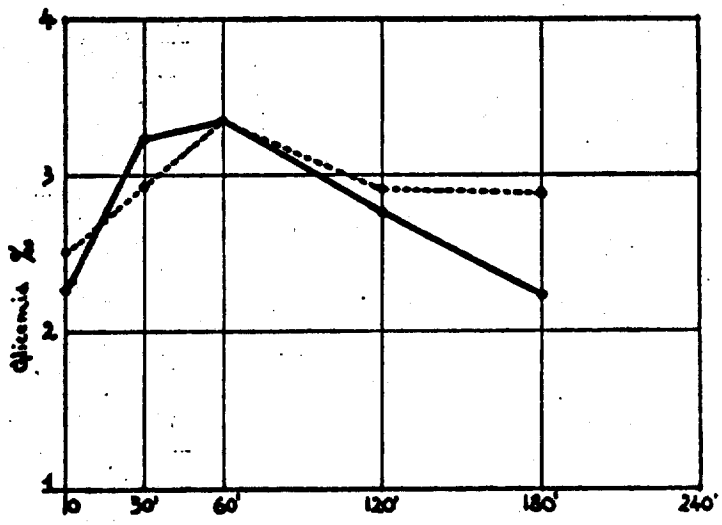

Fig. 2.

According to the data presented above, it appears that HCl has a definite hypoglycemic activity. It must be pointed out, however, that the reduction of blood sugar level is more marked in the fasting blood sugar

curve, as demonstrated by a decline of blood sugar level equal to 28%. On the contrary, glycosuria is affected only moderately. The hypoglycemic activity of HCl is also manifested following a low intensity insulin treatment (20 units per day), although at a less significant level.

CASE II. B. Luigi, 48 year old male, farmer. Mild diabetes mellitus.

For the past 2 months the patient complained of polyuria and gradual body weight loss (about 12 kg). Furthermore, he had severe asthenia and cutaneous pruritis, and had to quit his job. At the same time, polyuria became more severe (6 liters daily) and the patient was very hungry and thirsty. Heavy drinker of wine and beer. Denies any history of venereal diseases.

Examination: Acetone odor in the breath; advanced malnutrition. Slight hypophonesis at the level of the left supraspinal ? where respiration was relatively scarce. No other significant visceral manifestations. Surgical scar at the level of the left scrotal burse which is empty as a result of orchiectomy. Pmx: 175; Pmn: 80 (R.R.). Amount of urine: 3050 ml; Ps: 1037; Glucose: 52%; Acetone: present; Acetic acid: absent.

Glucose Tolerance Test
(50 g glucose in 250 ml of water)

	Glycemia %	Glycosuria %	Amount of urine	Total amount of glucose, in g
Fasting	2,02	0,62	290	0,18
After 0.5 hour	2,81	2,38	170	0,40
1	3,15	2,22	325	0,72
2	3,—	1,50	290	0,43
3	2,51	1,—	290	0,29
4	2,12	1,50	150	0,22
The following morning	—	6,30	1200	7.56
			2715	9,80

The same test following the administration of 100 ml of HCl officinalis
(50 g glucose in 250 ml of water)

	Glycemia %	Glycosuria %	Amount of urine	Total amount of glucose, in g
Fasting	1,72	—	55	—
After 0.5 hour	2,03	2,27	345	0,78
1	2,98	2,38	300	0,71
2	2,82	2,—	100	2,00
3	2,16	1,77	155	0,27
4	1,93	1,50	300	0,45
The following morning	2,13	5,—	1000	5,—
			2255	9,21

Fasting Blood Glucose Test

Without HCl	Glycemia %	Previous administration of 50 ml of a 0.50% HCl solution	Glycemia %
Fasting	2,45	Fasting	3,05
After 0.5 hour	2,39	After 0.5 hour	2,77
1	2,48	1	2,66
2	2,26	2	2,52
3	2,26	3	2,27

In this case also the administration of HCl resulted in a definite reduction of blood sugar, especially during the fasting blood sugar test (see Fig. 3).

CASE III. G. Maria, 32 year old female, married. Diabetes mellitus in an obese patient.

The patient complained of polydipsia, polyphagia, cutaneous and vulvar pruritis, progressive asthenia and body weight loss (about 11 kg in 6 months). The appearance of 20% glycosuria in 24 hour urine prompted the hospitalization of the patient.

Examination: Dental and alveolar pyorrhea. Visceral examination: negative. Pmx: 130; Pmn: 100 (R.R.). Urine amount: 1500-2000 ml; Ps: 1028; Glycosuria: 7%, Acetone: absent.

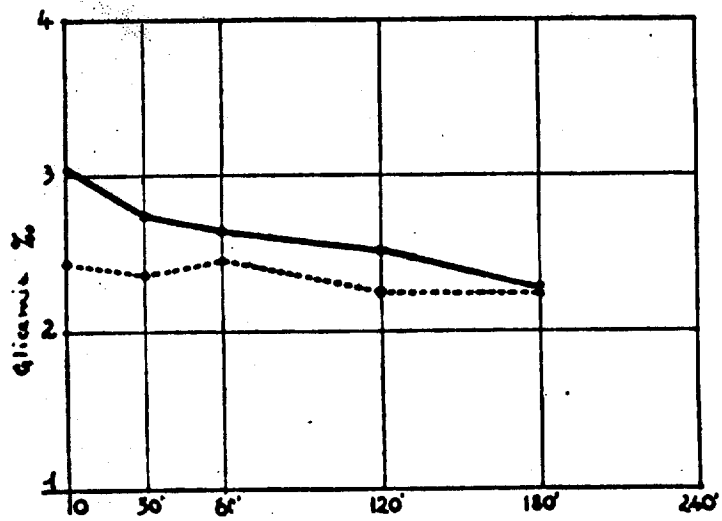

Fig. 3.

Fasting Blood Sugar Test

Without HCl	Glycemia %	Previous administration of 30 drops of HCl officinalis	Glycemia %
Fasting	1,62	Fasting	1,82
After 0.5 hour	1,47	After 0.5 hour	1,80
1	1,52	1	1,57
2	1,52	2	1,48
3	1,42	3	1,09
4	—	4	—

In this case also the hypoglycemic activity of HCl in the fasting patient is well evident.

CASE IV: P. Maria, 42 year old female; single, unemployed. Diabetes mellitus, right tuberculous gonitis.

At age 33, she had a fistula in the right knee. Treatment resulted in rapid improvement and disappearance of the fistula. One month before her admission to the clinic, the patient presented another fistula in the right knee, accompanied by pain upon movement. Moderate nocturnal thermal reactions. Asthenia, polydipsia and pollakiuria, but no polyphagia.

Examination: The right upper limb significantly shorter. Fluctuation of tissues around the peroneum; compression of the area induced sharp pain and the emergence of a thick white-yellowish pus from a fistula located in the postero-external portion of the leg, corresponding to the tendon of the femoral biceps. Visceral examination: negative. Pmx: 170; Pmn: 90 (R.R.). Amount of urine: 1200-1500 ml; Ps: 1035; Glucose: 25%; Acetone: absent.

The patient was treated with iodine and insulin. When tested for the effect of hydrochloric acid, glycosuria was fully absent.

Fasting Blood Sugar Test

Without HCl	Glycemia %	Previous administration of 50 ml of a 0.5% HCl solution	Glycemia %
Fasting	1,81	Fasting	1,60
After 0.5 hour	1,80	After 0.5 hour	1,45
1	1,76	1	1,37
2	1,70	2	1,36
3	1,72	3	1,31
4	1,75	4	1,24

CASE V: M. Serafina, 47 year old female, married; housewife. Diabetes mellitus in an overweight patient.

The diabetic condition appeared 2 years ago. The patient is overweight. Visceral examination: negative. Pmx: 125; Pmn: 60 (R.R.). Amount of urine: 1600 ml; Ps: 1078; Glucose: 10%; Acetone: absent.

Glucose Tolerance Curve
(50 g glucose in 250 ml of water)

	Glycemia %	Glycosuria %	Amount of urine	Total amount of glucose, in g
Fasting	2,30	4,16	45	0,18
After 0.5 hour	2,85	5,55	40	0,21
1	3,32	6,25	55	0,34
2	3,02	8,33	145	1,19
3	2,64	6,25	60	0,36
4	—	7,69	20	0,14
The following morning	—	14,—	800	11,20
			1165	13,62

The same test following the administration of 50 ml of a 0.5% HCl solution

	Glycemia %	Glycosuria %	Amount of urine	Total amount of glucose, in g
Fasting	2,24	2,—	70	0,14
After 0.5 hour	2,54	—	90	0,—
1	3,29	5,55	145	0,80
2	6,10	7,14	220	1,57
3	2,60	5,88	105	0,61
4	—	—	30	0,—
The following morning	—	9,—	950	8,55
			1610	11,67

Fasting blood sugar test, without HCl

	Glycemia %	Glycosuria %	Amount of urine	Total amount of glucose, in g
Fasting	1,80	—	65	—
After 0.5 hour	—	—	50	—
1	1,85	—	255	—
2	1,68	—	65	—
3	1,64	—	80	—
4	—	—	25	—
The following morning	—	9,—	790	7,11
			1330	7,11

Fasting blood sugar following the administration of 50 ml of a 0.5% HCl solution

	Glycemia %	Glycosuria %	Amount of urine	Total amount of glucose, in g
Fasting	2,17	2,63	65	0,16
After 0.5 hour	2,02	—	60	—
1	1,90	—	50	—
2	1,90	—	125	—
3	1,84	—	90	—
4	—	—	30	—
The following morning	—	7,14	630	4,50
			1050	4,66

From the results obtained in this patient, it emerges that HCl has definite hypoglycemic activity. In this particular case, the reduction of blood sugar levels is manifested particularly on the fasting blood sugar curve.

CASE VI: B. Maria, 59 year old female, widow. Tuberculotic infection; bilateral lung tuberculosis infiltrating the upper lobes; syphilis, ectatic aortitis; diabetes mellitus.

For the past year, the patient presented progressive body weight loss, anorexia, fatigue. For the past 2 months she presented cough with slight mucopurulent expectoration, nocturnal sweating and nocturnal fever.

Examination: Poor nutritional condition. Thorax: bilateral postero-superior plexus depression, mainly to the left. Reduction in the mobility of lower lung edges. Scarce breathing in the projection area of the upper lobes, especially to the left (supraspinal area) where auscultation revealed small and average rales. Increased projection area of the cardio-vascular fascia. Left ventricular hypertrophy. Pmx: 135; Pmn: 75 (R.R.) Urine: 1000 ml; Ps: 1028; Glucose: 20%, Wasserman and Meinicke reactions: +++. Koch bacillus present in the sputum.

Glucose Tolerance Curve
(50 g glucose in 250 ml water + 50 ml 0.5% HCl)

	Glycemia %	Glycosuria %	Amount of urine	Total amount of glucose, in g
Fasting	1,92	2,70	95	0,25
After 0.5 hour	2,39	2,50	45	0,11
1	2,96	9,10	20	0,18
2	3,07	5,—	75	0,37
3	2,57	20,—	45	0,90
4	—	3,60	45	0,16
The following morning	—	3,33	960	3,20
			1285	5,17

Fasting blood sugar in the absence of HCl

	Glycemia %	Glycosuria %	Amount of urine	Total amount of glucose, in g
Fasting	1,85	—	15	—
After 0.5 hour	1,79	—	25	—
1	1,70	—	25	—
2	1,59	—	25	—
3	1,55	—	30	—
4	—	—	30	—
The following morning	—	10,—	900	9,—
			1050	9,—

Fasting blood sugar following the administration of 50 ml of a 0.5% HCl solution

	Glycemia %	Glycosuria %	Amount of urine	Total amount of glucose, in g
Fasting	2,32	—	50	—
After 0.5 hour	2,14	—	30	—
1	1,96	—	30	—
2	1,78	—	40	—
3	1,64	—	15	—
4	—	—	45	—
The following morning	—	—	700	—
			910	—

In this case also the hypoglycemic activity of hydrochloric acid is well evident. The reduction of glycemia particularly manifest in the fasting blood sugar curve (see Fig. 4).

CASE VII: C. Cesare, 49 year old male, railroad worker, married. Diabetes mellitus.

The most significant disease history features were right inguinal hernia subjected to surgery at age 21; gonorrhea at age 34; symptoms of diabetes mellitus for the past 5 years. Received several courses of insulin.

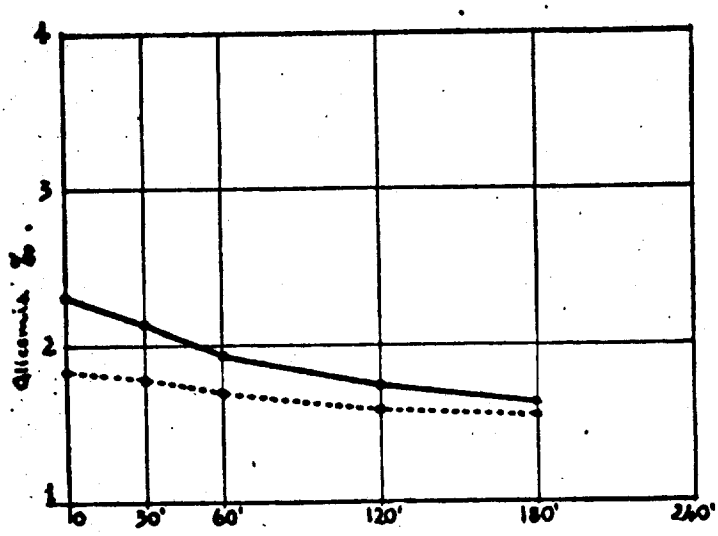

Fig. 4.

Examination: Pmx: 130; Pmn: 90 (R.R.). Amount of urine: 2000 ml; Ps: 1030; Glucose: 20%; Acetone and acetacetic acid: present. Visceral examination failed to reveal any abnormalities.

Glucose Tolerance Test
(50 g glucose in 250 ml water + 50 ml of 0.5% HCl)

	Glycemia %	Glycosuria %	Amount of urine	Total amount of glucose, in g
Fasting	2,46	25,—	25	0,63
After 0.5 hour	3,06	12,50	30	0,37
1	3,14	16,50	20	0,33
2	3,26	12,50	85	1,06
3	2,71	12,30	100	1,23
4	—	33,30	55	1,83
The following morning	—	30,—	2500	75,—
			2615	80,45

Fasting blood sugar in the absence of HCl

	Glycemia %	Glycosuria %	Amount of urine	Total amount of glucose, in g
Fasting	2,45	41,—	180	7,38
After 0.5 hour	2,46	42,—	35	1,47
1	2,39	33,—	30	0,99
2	2,46	46,—	90	4,14
3	2,32	25,—	50	1,25
4	—	20,—	40	0,80
The following morning	—	62,—	2480	153,76
			2905	169,79

Fasting blood sugar following the administration of 50 ml of a 0.5% HCl solution

	Glycemia %	Glycosuria %	Amount of urine	Total amount of glucose, in g
Fasting	2,64	25,—	200	5,—
After 0.5 hour	2,44	25,—	20	0,50
1	2,47	25,—	30	0,75
2	2,52	14,—	25	0,35
3	2,40	17,—	30	0,51
4	—	14,—	15	0,21
The following morning	—	33,—	1600	52,80
			1920	60,12

In this case, the administration of HCl failed to affect glycemia; even fasting blood sugar remained unchanged.

CASE VIII: M. Maria, 57 year old female; widow. Diabetes mellitus.

The diabetes appeared 7 years ago. Did not want to take insulin treatment.

Examination: Pmx: 140; Pmn: 70. Amount of urine: 2000 ml; Glucose: 25%. Examination of the viscera: negative.

Glucose Tolerance Test
(50 g glucose in 250 ml water - 50 ml of 0.5% HCl)

	Glycemia %	Glycosuria %	Amount of urine	Total amount of glucose, in g
Fasting	2,15	3,75	115	0,43
After 0.5 hour	2,65	1,—	35	0,035
1	3,11	8,30	60	0,49
2	3,08	16,60	25	0,41
3	2,92	12,50	40	0,50
4	—	14,—	20	0,28
The following morning	—	12,50	1750	21,87
			2045	24,015

Fasting blood sugar in the absence of HCl

	Glycemia %	Glycosuria %	Amount of urine	Total amount of glucose, in g
Fasting	2,10	2,7	55	0,14
After 0.5 hour	2,10	1,5	10	0,015
1	2,03	—	10	—
2	1,88	—	45	—
3	1,79	—	45	—
4	—	—	20	—
The following morning	—	6,2	1500	9,30
			1685	9,455

Fasting blood sugar following the administration of 50 ml of a 0.5% HCl solution

	Glycemia %	Glycosuria %	Amount of urine	Total amount of glucose, in g
Fasting	2,67	—	20	—
After 0.5 hour	2,67	—	15	—
1	2,45	—	30	—
2	2,27	—	125	—
3	2,14	—	90	—
4	—	—	10	—
The following morning	—	2,50	1000	2,50
			1290	2,50

In this case also, hydrochloric acid reduced fasting blood sugar levels but failed to affect the glucose tolerance curve.

Fasting Blood Glucose Test in Non-Diabetics

Disease	In absence of HCl Glycemia %					After the administration of 50 ml of a 0.5% HCl solution Glycemia %				
	prima	30'	1 h	2 h	3 h	prima	30'	1 h	2 h	3 h
Epilepsy	0,95	0,95	0,93	1,—	0,92	0,83	0,75	0,70	0,65	0,6[illegible]
Cerebral hemorrhage	0,90	092	0,98	0,96	0,94	0,93	0,94	1,06	0,87	1,—
Right tuberculotic gonitis	0,90	0,92	0,89	0,94	0,90	0,89	0,85	0,88	0,87	0,9[illegible]
Basedow-Fl disease	—	—	—	—	—	0,86	0,85	0,80	0,74	0,71
Neurosis	—	—	—	—	—	0,95	0,86	0,91	0,83	0,8[illegible]
Addison's disease	0,90	0,88	0,92	0,86	0,88	0,98	0,93	1,06	0,95	1,—

Results Obtained in Non-Diabetic Patients

The fasting blood sugar levels obtained in non-diabetic patients, before and after the oral administration of HCl, are summarized in the Table presented above.

A general examination of the blood sugar curves described earlier indicates that the oral administration of hydrochloric acid has a definite effect on glycemia.

Various degrees of decline in blood sugar levels were found in almost all diabetic patients tested; the reduction of blood sugar was generally evident at one to two hours subsequent to the ingestion of the hydrochloric acid solution.

However, the shape of the glucose tolerance curve was not completely similar to that of the fasting blood sugar curve. Actually, while the administration of HCl resulted in a significant reduction of fasting glycemia (by 28%), treatment failed to inhibit glucose induced glycemia in all but one patient (in the latter, the blood glucose levels at 3 hours

after the administration of HCl practically returned to normal.

Examination of fasting blood sugar levels in the absence of HCl demonstrated that fasting diabetic glycemia might decline spontaneously. This observation was also made by other investigators and, while it must be taken into account during the evaluation of the effect induced by HCl, it does not necessarily disagree with our findings since the spontaneous reduction of fasting blood sugar levels was found to be very slight.

In the non-diabetic individual, hydrochloric acid had a very slight effect on blood sugar. This phenomenon was also reported by Zunz and La Barre during their control studies in the normal dog. The explanation for the lack of hypoglycemic activity of HCl in the normal individual is that the fasting blood glucose levels in the latter vary very little if at all, even during long term fasting. In his studies regarding the effect of neurohormonal factors in the pathogenesis of diabetes mellitus, Moretti found that fasting blood sugar of normal individuals is not affected by ergotamine (an alkaloid with significant hypoglycemic action).

The results of the present study also confirm the recent experimental research carried out by Zunz and La Barre who found that the introduction of HCl in the duodenum of adrenalectomized dogs induces a certain decrease of blood sugar levels.

According to Gley et al., this particular phenomenon is related to insulin hypersecretion induced by secretin. Actually, Gley encountered a hypoglycemic activity in the serum obtained from the pancreato-duodenal vein of chloralosed dogs treated intraduodenally with HCl. Hypoglycemia was also identified in animals whose splenic vein was anastomized with the pancreato-duodenal vein of donor animals treated intraduodenally with a hydrochloric acid solution. According to Gley and Hazard, the passage

of the pancreatic venous blood of a given animal into the circulation of another animal of the same species suffices to induce reduction of blood sugar levels. However, this response is even more remarkable following the introduction of HCl in the duodenum since the acid results in abundant pancreatic secretion and formation of secretin.

On the other hand, it has been reported that hypoglycemia subsequent to the injection of secretin is not only the consequence of an increase of the endocrine pancreatic secretion. As a matter of fact, Zunz and La Barre in their experiments with the pancreato-jugular anastomosis, made the observation that the levels of glycemia also decline gradually in the donor animal, whose pancreatic venous blood is transfused to another animal. Furthermore, Takacs used secretin to obtain significant reduction of blood sugar in pancreatectomized dogs.

Nevertheless, the hypothesis claiming that secretin-induced hyperinsulinemia is the main factor responsible for the hypoglycemic activity of hydrochloric acid is also supported by the experiments of Dale who determined an increase in islet tissue in the secretin-treated dog. Furthermore, Fieschi was able to increase by as much as twofold the carbohydrate tolerance of animals (dogs) fed a predetermined constant diet and injected for 30 days with secretin obtained by the method of Bayliss and Starling. The animals displayed the same degree of hypoglycemia for the entire duration of the experiment (from 1.0 to 1.5 hours following the injection of secretin).

Earlier, I mentioned that Epstein et al. demonstrated that insulin is antagonized by trypsin , both in vivo and in vitro. This particular phenomenon has been attributed to the formation of a compound between insulin and trypsin.

According to Epstein, under certain predetermined conditions, the pancreatic capillary blood vessels become permeable to the products of acinary secretion, especially to trypsin and even more so to protrypsin and trypsinogen. Under these circumstances, Grott claims that insulin is neutralized or immobilized, while the passage of protrypsin in portal circulation results in hepatic glycogenolysis and subsequent hyperglycemia and also glycosuria. According to Epstein, this is the mechanism through which trypsin contributes to the regulation of the endocrine pancreatic secretion. When the islets secrete excessive amounts of insulin, a well known secretion stimulating agent, the passage of trypsin (or of trypsinogen) into the blood originating from the islets contributes to the prevention of the damaging effects of hypoglycemia. Furthermore, according to Epstein, the intravenous injection of insulin into a pancreato-duodenal vein, instead of producing hypoglycemia, in the cat, induces hyperglycemia and glycosuria due to the passage of protrypsin in the venous blood. As opposed to this, the introduction into the pancreato-duodenal artery of insulin mixed with a solution of safranin (a dye that stains the zymogenic granulation of the acini very well) does not fail to produce either hyperglycemia or glycosuria. In an attempt to evaluate this phenomenon, Epstein hypothesized that the combination between trypsin and dye is highly stable and, therefore, it obstructs the passage of trypsinogen into the venous blood of the islands and induces significant neutralization of insulin.

According to the opinions of Epstein, reduction of the amount of protrypsin in the acini should result in a more marked activity of insulin in the organism. Therefore, it can be assumed that the stimulation of exocrine pancreatic secretion, either with HCl or with secretin, reduces the amount of protrypsin available in the pancreas by sending it into the intestine;

at the same time, the amount of insulin entering the circulation increases, and therefore blood sugar level declines.

According to Dixon and Hamill, and also Hustin, secretin is easily captured by the secretory elements of the glandular acini. On the basis of this particular finding, it can be hypothesized that the stimulation of the hypoglycemic activity of secretin by hydrochloric acid depends on the reduction of the amount of trypsin which, at least in vitro, is capable of neutralizing insulin. However, many objections have been raised as to the validity of the hypothesis formulated by Dixon and Hamill, and by Hustin, in reference to the exocrine secretion of the pancreas. Epstein's theory regarding the effect of trypsin on the regulation of glycemia has also encountered severe criticism. Furthermore, the explanation offered by Zunz and La Barre regarding the action mechanism of secretin on glycemia remains purely theoretical, especially since Takacs made the observation that secretin has a hypoglycemic action even in pancreatomized animals.

In addition to the action mechanism of secretin on the endocrine pancreatic secretion, there is the problem of the mechanism responsible for the reduction of blood sugar levels accompanying the exocrine pancreatic secretion following the administration of HCl. We are in no position to conclude that this particular phenomenon is due merely to the formation of secretin. Meyer, Bisch, Bock and Wohlemberg, and Martino also, claim to have isolated an anti-insulin hyperglycemic factor from the exocrine human pancreatic secretion. According to these investigators, the injection of an ultrafiltration product from the pancreatic juice or the oral administration of pancreatic juice increases the blood sugar level in both rabbits and dogs.

According to these findings and as already hypothesized by Zunz and La Barre, the decline in blood sugar levels could also depend on the

antagonizing effect of this hyperglycemia-inducing principle on insulin, both in the pancreas and in the circulating blood. However, this problem can be elucidated only by further research. As of now, the only concrete finding remains the hypoglycemic activity of HCl reported by Usuelli. In 1927, this investigator determined that pancreas hyperemia induced by secretin or by the duodenal administration of HCl induces hyperactivity of the islet areas, in addition to activating the exocrine pancreatic secretion.

In the diabetic patient, the administration of HCl generally induces a beneficial, although transient effect on blood sugar levels. This finding has only a moderate practical significance, although its theoretical consequences are quite interesting since it calls for studies of the gastric secretion and of exocrine pancreatic secretion in the diabetic patient. Actually, many studies have been carried out on the gastric secretion of diabetics. The results of these studies indicate the presence of frequent hypo-anachlorydria (25% according to Joslin, 32% according to Wiechmann and Elzas, and 89% according to the recent studies of Rabinovich, Fowler and Vatson).

Chiray, Salmon, Mercier, Gavrila and Paraschivesco, and others, have shown that diabetics also manifest reduced exocrine pancreatic secretion.

All of these questions can be elucidated only by systematic research, the only concrete feature emerging from the various studies being the existence of a relationship between gastric secretion and the endocrine pancreatic function.

These observations lead to the conclusion that the pathogenesis of diabetes mellitus must be considered not only in the light of the basic "pancreatic insufficiency" element but also from that of the "gastric insufficiency" factor.

Summary

Oral administration of hydrochloric acid to diabetic patients induces practically uniformly various degrees of reduction in blood sugar levels (20-30%).

The hypoglycemic activity of HCl is particularly manifest during fasting.

In individuals free of digestive pathology, blood sugar levels are only slightly affected by the administration of hydrochloric acid.

Minerva Med. 22-II(34):245-253. 1931.

Boattini-'31
Bib. no. 40

LAVORI ORIGINALI

Istituto di Patologia Spec. Medica della R. Univ. di Milano
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L'azione ipoglicemizzante dell'acido cloridrico nel diabete mellito

Dott. Giorgio Boattini, *Assistente*

I rapporti tra le differenti attività funzionali del tessuto acinoso e del tessuto insulare del pancreas hanno sempre costituito la base fondamentale dello studio della patogenesi del diabete mellito. Infatti, da quando furono invocate come causa di tale malattia le alterazioni di tutti i vari elementi citologici costitutivi del pancreas, e da quando fu avanzata l'ipotesi che il diabete umano avesse a fondamento una alterazione del tessuto insulare, è stato tutto un susseguirsi di indagini sino alla scoperta della insulina, con la quale ha trionfato, nella patogenesi del diabete pancreatico, la teoria ormonica sulle altre teorie, che le avevano conteso il campo: la nervosa e la autotossica. Ma ancora è dibattuta la questione se le isole di Langherans rappresentino formazioni sicuramente indipendenti dagli acini pancreatici o se invece possano derivare, nell'individuo a completo sviluppo, dagli acini ed ancora se di esse sia possibile la metaplasia in acini. Questa seconda opinione, che come è risaputo fu recisamente negata da valenti studiosi italiani, è stata invece difesa dal Laguesse che su di essa ha fondato la teoria del « balancement » degli elementi cellulari pancreatici, secondo la quale tra i due tessuti, endocrino ed esocrino, dovrebbe ammettersi una continua reversibilità morfologica e quindi funzionale. E in appoggio delle vedute del Laguesse, della possibile formazione cioè degli isolotti a spese degli acini, parlerebbero anche recenti studi anatomo-comparativi di Bierry e di Kollmann.

Benchè si discuta ancor oggi sulla perennità o meno del tessuto insulare, un fatto ormai risulta stabilito: l'origine comune delle cellule insulari e delle cellule degli acini dagli epiteli dei tubuli pancreatici primitivi. Questa conoscenza istogenetica, nonostante la scoperta dell'insulina, ha fatto perdere alquanto della sua rigidità alla teoria dualista della fisiologia pancreatica, tanto più dopo che questa si è arricchita di nuove conoscenze.

E' stato dimostrato, ad esempio, per opera dell'Epstein, del Rosenthal, del Maechling, del De Bek, del Grott e del Monteleone, che l'insulina sia inattivata in vitro ed in vivo dalla tripsina, come del resto già avevano osservato Banting e Best, e che l'insulina sia a sua volta dotata di potere succagogo. Il Deusch ed il Drost, come pure altri studiosi, riuscirono infatti a provocare con iniezioni di insulina un notevole aumento della secrezione pancreatica esterna nell'uomo e negli animali da esperimento (cane). Il Martino inoltre sarebbe riuscito ad isolare dal secreto esterno del pancreas una sostanza iperglicemizzante; reperto questo di particolare interesse poichè dimostrerebbe l'influenza del tessuto acinoso nel meccanismo regolatore dello zucchero ematico. L'azione di questa sostanza iperglicemizzante sarebbe risultata anche manifesta in animali (cani) precedentemente tenuti a digiuno per alcuni giorni e nei quali era stata poi provocata secrezione pancreatica mediante alimentazione con sostanze proteiche. Il Ciaccio ed il Racchiusa nei cani digiunanti da 24 - 48 ore hanno pure osservato tale fenomeno, che poteva essere anche provocato dalla somministrazione per os di HCl e col pasto psichico.

Risultando adunque il secreto pancreatico esterno capace di influenzare la glicemia, i ricercatori sono stati logicamente indotti a studiare se le sostanze attivatrici della secrezione pancreatica esterna (secretina ed HCl) fossero capaci di influenzare anche la secrezione interna della ghiandola.

Dopo la scoperta della secretina per opera di Bayliss e Starling nel 1902, si è a più riprese cercato di vedere se tale sostanza iniettata endovena e sottocute provocasse modificazioni della glicemia.

Il Pariset, il Mellamby ed il Delezenne non poterono rilevare alcuna modificazione del tasso glicemico. L'Abelous ed il Soula, il Lambert e l'Hermann notarono iperglicemia transitoria seguita da modesta ipoglicemia. Il Troteano, l'Oehme e il Wimmers, il Penau e il Simonnet, il Novao Santos, il Fieschi, il Takacs, segnalarono invece diminuzione dello zucchero sanguigno tanto nel coniglio e nel cane come nell'uomo sano e diabetico. Queste discordanze sembrano legate in gran parte al grado di purificazione della secretina.

Lo Zunz ed il La Barre, in base a numerose ricerche, sono stati indotti a ritenere non attendibili i risultati del Pariset ed hanno invece confermato quelli del Denjab e del Mellamby che osservarono attiva secrezione pancreatica iniettando nella vena porta e nelle vene femorali e giugulari la secretina purificata. E' da ricordare che l'estratto intestinale non sempre veniva però privato delle sostanze vasodepressive e specialmente della istamina, la quale come è noto provoca una considerevole iperglicemia, cui fa seguito un rapido ritorno a valori normali come ebbero a dimostrare lo Chambers ed il Thompson, il Monteleone, il Carusi e numerosi altri studiosi. Il La Barre inoltre mediante l'anastomosi surreno-giugulare nel cane avrebbe osservato un brusco aumento del tasso adrenalinico e perciò iperglicemia nel sangue venoso surrenale dopo introduzione endovena di istamina.

Di questa sostanza sarebbe stata trovata dal Parson una percentuale di circa il 2 °/₀₀ nelle secretine non purificate. Tale constatazione ha indotto qualche studioso, il Guggenheim ad esempio, ad identificare la secretina con l'istamina. Secondo il Popielski ed il

Takacs inoltre, solo a quest'ultima dovrebbe essere riconosciuto il potere succagogo degli estratti intestinali. Ma ulteriori studi del Bayliss e dello Starling, del Dixon e dell'Hamill, del Divry, dello Stepp, del Luckhardt, del Barlow, del Weaver e del Koch, ecc., hanno dimostrato che secretina ed istamina sono due sostanze nettamente differenti. Lo Zunz e il La Barre, infine, impiegando varie secretine ottenute col metodo di Still, di Mellamby e di Underhill (secretine private di potere ipotensivo) hanno potuto chiaramente documentare l'alto potere succagogo di tali sostanze, nel contempo dotate di azione ipoglicemizzante. E l'azione ipoglicemizzante non sarebbe risultata dipendente dalla presenza di insulina nelle secretine, come riteneva il Mellamby, poichè gli studiosi ora ricordati ottennero diminuzione del tasso glicemico degli animali in esperimento anche con iniezioni di secretina precedentemente trattata con soluzioni acide di pepsina.

Da lungo tempo è noto che l'acido cloridrico del chimo che arriva in duodeno rappresenta il principale fattore eccitante della secrezione pancreatica. Vivaci sono state le discussioni per stabilire attraverso quale meccanismo agisse l'HCl: se cioè questo esplicasse la sua azione per via nervosa riflessa o per via umorale.

Si è riconosciuto che l'eccitamento del simpatico e del vago provoca secrezione pancreatica; recenti esperienze dello Zunz, del La Barre e del Destrée ne hanno dato infatti la definitiva dimostrazione. Ma è pure noto che il secreto pancreatico compare sotto l'azione dell'HCl introdotto in duodeno anche dopo la sezione dei due vaghi e dei due simpatici, dopo la separazione del bulbo dal midollo ed anche dopo la asportazione del plesso solare e di quello mesenterico superiore, come ebbero a dimostrare sin dal 1899-1901 il Werteimer ed il Lepage e recentemente a conferma il Czarnecki. All'ora attuale, dopo le classiche esperienze di Bayliss e Starling, confermate da una larga schiera di studiosi, domina la teoria umorale della azione succagoga dell'HCl. L'acido cloridrico forma cioè secretina dotata, come abbiamo visto, di alto potere succagogo sul pancreas, anche quando questo sia stato completamente isolato da ogni connessione nervosa.

Basandosi su tali conoscenze Freud e Saadi-Nazim, ispirati da Gley, che fu pure sostenitore dell'esistenza di intimi rapporti funzionali fra tessuto acinoso e tessuto insulare, preferirono affrontare il problema della influenza della secrezione pancreatica esterna su quella interna, anzi che ricorrendo ad estratti di mucosa intestinale qualificati come secretina, a sostanze quindi artificialmente preparate, eccitando invece la secrezione pancreatica in condizioni assai vicine a quelle fisiologiche, introducendo cioè una soluzione di acido cloridrico nel duodeno. Questi studiosi poterono così osservare nel cane una diminuzione del tasso glicemico che iniziava generalmente 15 - 30 minuti dopo la introduzione di HCl e proseguiva per 1 - 2 ore; le modificazioni della glicemia erano accompagnate da aumentata secrezione pancreatica.

Coelho e Oliveira ripeterono tali esperienze nell'uomo non affetto da disturbi del tubo gastro-enterico, del pancreas e del fegato. Mediante la sonda di Einhorn introducevano 30 cc. di HCl $^{n}/_{10}$; prelevavano quindi a più riprese il succo pancreatico di cui dosavano la tripsina e l'amilasi e determinavano contemporaneamente il tasso glicemico.

Su 20 degli individui studiati, 14 presentarono un abbassamento della glicemia mezz'ora dopo l'introduzione dell'HCl; in quattro casi il tasso glicemico non subì alcuna modificazione; in due altri questo aumentò leggermente nel primo quarto d'ora. L'aumento della secrezione pancreatica esterna venne documentata col dosaggio dei fermenti. Tali reperti condussero gli AA. ora citati a ritenere che la eccitazione della secrezione esterna del pancreas provocasse contemporaneamente un aumento della secrezione interna e cioè uno stato di iperinsulinemia, rappresentato dalla diminuzione del tasso glicemico. Devo ricordare che tale meccanismo fisiologico della insulinemia durante la secrezione pancreatica esterna era già stata prospettata da Gley e da Hazard. Questi AA. infatti nel cane, la cui giugulare era stata anastomizzata colla vena splenica di un altro cane, nel duodeno del quale veniva iniettata una soluzione di HCl, avevano potuto riscontrare diminuzione della glicemia del 20 % del valore iniziale.

Recentissime sono infine le esperienze di Zunz e La Barre (novembre 1930) che hanno condotto a risultati di particolare interesse. La somministrazione di HCl a cani normali cloralosati ed a cani surrenalectomizzati e cloralosati determinò costante diminuzione più o meno marcata del tasso glicemico negli animali scapsulati, mentre riuscì del tutto inattiva negli animali normali. In un secondo gruppo di esperimenti Zunz e La Barre praticarono l'anastomosi pancreatico-giugulare tra due cani scapsulati e cloralosati. Nell'animale datore, al quale veniva praticata l'iniezione duodenale di HCl, la secrezione pancreatica era assai modesta, mentre si riscontrava diminuzione del tasso glicemico che era particolarmente spiccata nell'animale ricevitore anche quando questo era stato vagotomizzato.

Dagli studi dello Zunz e del La Barre risulterebbe adunque ancor più documentata la correlazione funzionale tra tessuto esocrino ed endocrino del pancreas ed ancora appare la grande importanza di uno dei componenti del chimo gastrico, l'HCl, sulla glicoregolazione. Ma poichè questi AA., come fisiologi, hanno eseguito le loro indagini solo sugli animali, ho ritenuto opportuno ricercare se anche nell'uomo affetto da diabete mellito, oltre che nell'uomo indenne da disturbi dell'apparato digerente, il tasso glicemico venisse influenzato dalla somministrazione per os di acido cloridrico.

* * *

Le ricerche vennero condotte su otto malati di diabete mellito di diversa gravità. L'azione dell'acido cloridrico venne saggiata sia a digiuno che durante

la glicemia alimentare. Furono pure eseguite ricerche in soggetti non diabetici. I dosaggi dello zucchero vennero eseguiti col metodo di Bang-Cruto.

Ricerche sui diabetici

Caso I. — R. Maria, a. 59, vedova. *Diabete mellito di media gravità in soggetto arteriosclerotico e polisarcico.*

Da 4 mesi polidipsia, poliuria e pollachiuria; non polifagia inizialmente. Progressiva astenia e rapido deperimento (in 5 mesi l'a. sarebbe diminuita di circa 10 Kg. di peso). Essendosi riscontrata glicosuria ricovera in Clinica.

E. O. — Soggetto polisarcico. Piorrea dentaria alveolare. Negativo l'esame viscerale; lievi note artritiche delle ginocchia e dell'articolazione tibio-tarsica di sinistra. Pmx 150, Pmn 80 (RR.). Urine 2500 cc.; Ps. 1033; Glucosio 20 ‰; Acetone traccie; acido diacetico assente.

Curva della glicemia alimentare

(gr. 50 di glucosio in cc. 250 di acqua)

	Glicemia ‰	Glicosuria ‰	Quantità urine	Quant. tot. glucosio gr.
A digiuno	2,50	0,70	185	0,13
½ ora dopo	2,91	1,44	90	0,13
1 » »	3,36	0,61	220	0,13
2 » »	2,90	0,63	340	0,21
3 » »	2,88	1,08	275	0,30
4 » »	—	0,68	240	0,16
Al mattino successivo	—	0,23	1350	0,31
			2700	1,37

La stessa prova previa somministrazione di goccie 100 di HCl officinale.

	Glicemia ‰	Glicosuria ‰	Quantità urine	Quant. tot. glucosio gr.
A digiuno	2,27	1,03	150	0,15
½ ora dopo	3,23	5,60	60	0,33
1 » »	3,35	0,50	200	0,10
2 » »	2,78	0,50	200	0,10
3 » »	2,22	1,11	180	0,20
4 » »	—	2,—	75	0,15
Al mattino successivo	2,40	0,23	1500	0,34
			2365	1,37

Prova della glicemia a digiuno

Senza somministrazione di HCl Glicemia ‰			Previa somministrazione di goccie 50 di HCl officinale Glicemia ‰		
A digiuno	2,61	2,53	A digiuno	2,68	2,25
½ h dopo	2,61	2,57	½ h dopo	2,53	2,15
1 » »	2,61	2,58	1 » »	2,15	2,14
2 » »	2,67	2,57	2 » »	2,20	1,82
3 » »	2,57	2,53	3 » »	2,09	1,72
4 » »	—	2,50	4 » »	—	1,50

Le prove della glicemia a digiuno previa somministrazione di acido cloridrico vennero anche eseguite dopo che la p. era stata sottoposta a trattamento insulinico. Le iniezioni di insulina vennero sospese 24 ore prima di ciascuna prova. Riporto un protocollo.

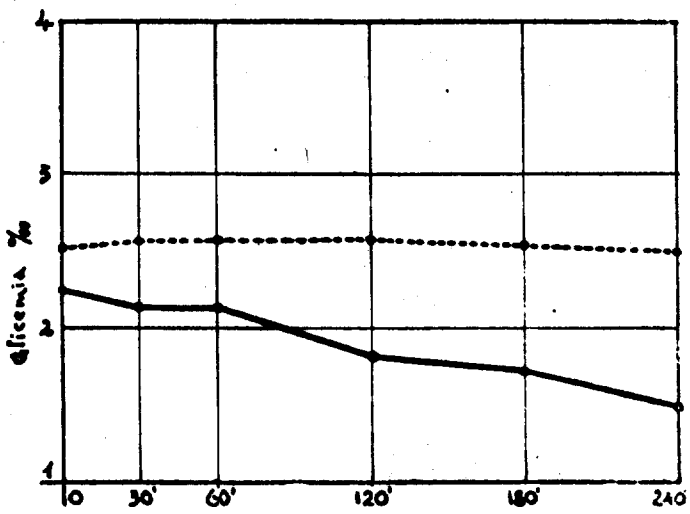

Fig. 1

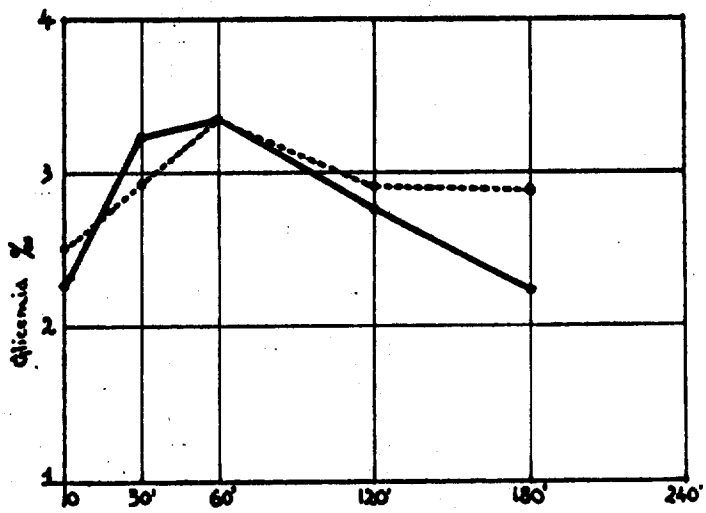

Fig. 2

Prova della glicemia a digiuno

Senza somministrazione di HCl Glicemia ‰		Previa somministrazione di cc. 50 di soluzione all'1 % di HCl Glicemia ‰	
A digiuno	1,90	A digiuno	1,77
½ h dopo	1,88	½ h dopo	1,67
1 » »	1,88	1 » »	1,60
2 » »	1,80	2 » »	1,43
3 » »	1,76	3 » »	1,47

Dai dati sopra riportati risulta adunque netta l'azione ipoglicemizzante dell'HCl. E' da rilevare però che la riduzione del tasso glicemico appare specialmente manifesta dalla curva glicemica a digiuno, nella quale si è potuto riscontrare una diminuzione del 28 % del tasso glicemico. Modesta è stata invece l'azione sulla curva glicosurica. Anche dopo trattamento insulinico, poco intenso (20 unità pro die), l'HCl ha esplicato, sebbene in modo meno marcato, la sua azione ipoglicemizzante.

Caso II. — B. Luigi, a. 48, contadino. *Diabete mellito magro.*

Da due mesi l'a. accusa poliuria e progressivo deperimento

(è diminuito di circa 12 Kg. di peso); accusa inoltre intensa astenia e prurito cutaneo; perciò fu costretto ad abbandonare le sue occupazioni. Contemporaneamente si intensificò la poliuria (sei litri pro die); sete e fame vivissime. Forte bevitore di vino e di birra; nega malattie veneree.

E. O. — Alito acetonico, notevole denutrizione. Lieve ipofonesi a livello della sopraspinosa sin. ove il respiro è piuttosto scarso. Null'altro a carico dei visceri. Cicatrice operatoria a livello della borsa scrotale sin. che appare vuota (orchiectomia). Pmx 175, Pmn 80 (R.R.) Quantità urine 3050; Ps. 1037; Glucosio 52 ‰; Acetone presente; Acido acetacetico assente.

Curva della glicemia alimentare
(gr. 50 di glucosio in cc. 250 di acqua)

	Glicemia ‰	Glicosuria ‰	Quantità urine	Quant. tot. glucosio gr.
A digiuno	2,02	0,62	290	0,18
½ ora dopo	2,81	2,38	170	0,40
1 » »	3,15	2,22	325	0,72
2 » »	3,—	1,50	290	0,43
3 » »	2,51	1,—	290	0,29
4 » »	2,12	1,50	150	0,22
Al mattino successivo	—	6,30	1200	7.56
			2715	9,80

La stessa prova previa somministrazione di goccie 100 di HCl officinale.

	Glicemia ‰	Glicosuria ‰	Quantità urine	Quant. tot. glucosio gr.
A digiuno	1,72	—	55	—
½ ora dopo	2,03	2,27	345	0,78
1 » »	2,98	2,38	300	0,71
2 » »	2,82	2,—	100	2,00
3 » »	2,16	1,77	155	0,27
4 » »	1,93	1,50	300	0,45
Al mattino successivo	2,13	5,—	1000	5,—
			2255	9,21

Prova della glicemia a digiuno

Senza somministrazione di HCl Glicemia ‰		Previa somministrazione di cc. 50 di soluzione al 0,50 % di HCl Glicemia ‰	
A digiuno	2,45	A digiuno	3,05
½ h dopo	2,39	½ h dopo	2,77
1 » »	2,48	1 » »	2,66
2 » »	2,26	2 » »	2,52
3 » »	2,26	3 » »	2,27

Anche in questo caso netta appare l'azione ipoglicemizzante dell'HCl, specialmente nella prova della glicemia a digiuno (vedi fig. 3).

Caso III. — G. Maria, a. 32, coniugata. *Diabete mellito in polisarcica.*

Polidipsia, polifagia, prurito cutaneo e vulvare, progressiva astenia e diminuzione di peso (avrebbe perduto circa 11 Kg. di peso in sei mesi). Essendo stata riscontrata glicosuria del 20 ‰ su urine delle 24 ore, la p. ricovera in Clinica.

E. O. — Piorrea dentaria ed alveolare. Negativo l'esame viscerale. Pmx 130, Pmn 100 (RR.). Quantità urine cc. 1500-2000; Ps. 1028; Glicosuria 7 %; Acetone assente.

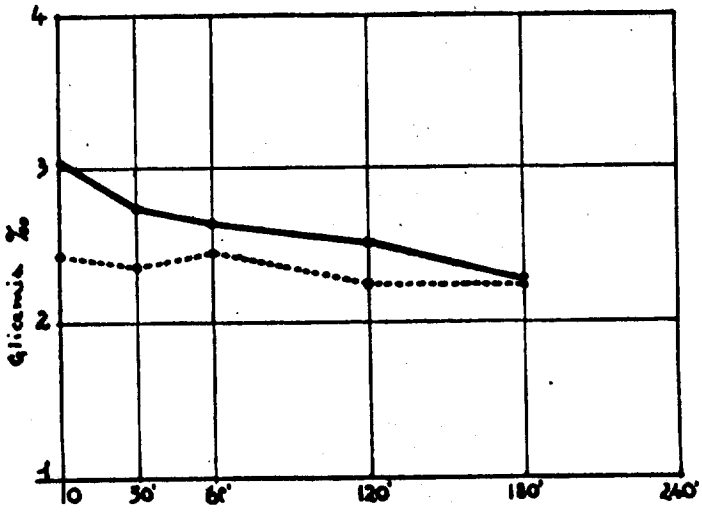

Fig. 3

Prova della glicemia a digiuno

Senza somministrazione di HCl Glicemia ‰		Previa somministrazione di goccie 30 di HCl officinale Glicemia ‰	
A digiuno	1,62	A digiuno	1,82
½ h dopo	1,47	½ h dopo	1,80
1 » »	1,52	1 » »	1,57
2 » »	1,52	2 » »	1,48
3 » »	1,42	3 » »	1,09
4 » »	—	4 » »	—

E' pure evidente in questo caso l'azione ipoglicemizzante dell'HCl a digiuno.

Caso IV. — P. Maria, a. 38, nubile, casalinga. *Diabete mellito, gonilite tubercolare destra.*

A 33 anni sofferse di ascesso freddo fistolizzato del ginocchio destro. In seguito alle cure si ebbe rapida chiusura della fistola. Un mese prima del suo ricovero in Clinica è comparsa una nuova fistola nel ginocchio destro; vivissimi dolori nei movimenti dell'arto. Modesti rialzi termici notturni. Astenia, polidipsia e pollachiuria; non polifagia.

E. O. — Arto superiore destro notevolmente accorciato; il ginocchio è tumefatto. Fluttuazione in corrispondenza dei tessuti sovrastanti la testa del perone: la pressione su tale regione provoca vivo dolore e fuoriuscita di pus denso biancogiallastro da una fistola situata nella porzione postero-esterna dell'estremità inferiore della gamba, in corrispondenza del tendine del bicipite femorale. Negativo l'esame viscerale. Pmx 170, Pmn 90 (R.R.). Quantità urine cc. 1200-1500; Ps 1035; Glucosio 25 ‰; Acetone assente.

La p. è stata sottoposta a cure iodiche e a trattamento insulinico. Quando vennero istituite le prove con l'acido clorcidrico, era del tutto cessata la glicosuria.

Prova della glicemia a digiuno

Senza somministrazione di HCl Glicemia ‰		Previa somministrazione di cc. 50 di soluzione al 0,5 % di HCl Glicemia ‰	
A digiuno	1,81	A digiuno	1,60
½ h dopo	1,80	½ h dopo	1,45
1 » »	1,76	1 » »	1,37
2 » »	1,70	2 » »	1,36
3 » »	1,72	3 » »	1,31
4 » »	1,75	4 » »	1,24

Caso V. — M. Serafina, a. 47, coniugata, casalinga. *Diabete mellito in polisarcica.*

Da due anni sofferente di diabete. Soggetto polisarcico. Negativo l'esame viscerale. Pmx 125, Pmn 60 (R.R.). Quantità urine 1600; Ps 1078; Glucosio 10 ‰; Acetone assente.

Curva della glicemia alimentare

(Gr. 50 di glucosio in cc. 250 di acqua)

	Glicemia ‰	Glicosuria ‰	Quantità urine	Quant. tot. glucosio gr.
A digiuno	2,30	4,16	45	0,18
½ ora dopo	2,85	5,55	40	0,21
1 » »	3,32	6,25	55	0,34
2 » »	3,02	8,33	145	1,19
3 » »	2,64	6,25	60	0,36
4 » »	—	7,69	20	0,14
Al mattino successivo	—	14,—	800	11,20
			1165	13,62

La stessa prova previa somministrazione di cc. 50 di HCl al 0,5 %.

	Glicemia ‰	Glicosuria ‰	Quantità urine	Quant. tot. glucosio gr.
A digiuno	2,24	2,—	70	0,14
½ ora dopo	2,54	—	90	0,—
1 » »	3,29	5,55	145	0,80
2 » »	3,10	7,14	220	1,57
3 » »	2,60	5,88	105	0,61
4 » »	—	—	30	0,—
Al mattino successivo	—	9,—	950	8,55
			1610	11,67

Prova della glicemia a digiuno senza somministraz. di HCl

	Glicemia ‰	Glicosuria ‰	Quantità urine	Quant. tot. glucosio gr.
A digiuno	1,80	—	65	—
½ ora dopo	—	—	50	—
1 » »	1,85	—	255	—
2 » »	1,68	—	65	—
3 » »	1,64	—	80	—
4 » »	—	—	25	—
Al mattino successivo	—	9,—	790	7,11
			1330	7,11

Prova della glicemia a digiuno

previa somministrazione di cc. 50 di HCl al 0,5 %

	Glicemia ‰	Glicosuria ‰	Quantità urine	Quant. tot. glucosio gr.
A digiuno	2,17	2,63	65	0,16
½ ora dopo	2,02	—	60	—
1 » »	1,90	—	50	—
2 » »	1,90	—	125	—
3 » »	1,84	—	90	—
4 » »	—	—	30	—
Al mattino successivo	—	7,14	630	4,50
			1050	4,66

Dai risultati riferiti risulta chiara l'azione ipoglicemizzante dell'HCl. In questo caso la diminuzione del tasso glicemico si è resa manifesta soltanto nella curva glicemica a digiuno.

Caso VI. — B. Maria, a. 59, vedova. *Infezione tubercolare, tubercolosi polmon. bilaterale a tipo infiltrativo dei lobi superiori; infezione luetica: aortite ectasiante; Diabete mellito.*

Da un anno progressivo deperimento, scarso appetito, facile esauribilità. Sofferente da due mesi di tosse con discreto espettorato muco-purulento, sudori notturni e rialzi termici serotini.

E. O. — Nutrizione scaduta. Torace: smorzatura plessica bilaterale postero-superiore prevalentemente a sinistra. Riduzione della mobilità dei margini polmonari inferiori. Respiro scarso sull'aia di proiezione dei lobi sup. specie a S. (regione sopra-spinosa) ove si ascoltano rantoli a piccole e medie bolle. Aumento dell'aia di proiezione del fascio cardiovascolare. Ipertrofia del ventricolo sinistro. Pmx 135, Pmn 75 (R.R.). Urine 1000 cc.; Ps 1028; Glucosio 20 ‰. Reazioni di Wassermann e di Meinicke + + +. Bacillo di Koch presente nello sputo.

Curva della glicemia alimentare

(Gr. 50 di gluc. in cc. 250 di acqua + cc. 50 di HCl al 0,5 %)

	Glicemia ‰	Glicosuria ‰	Quantità urine	Quant. tot. glucosio gr.
A digiuno	1,92	2,70	95	0,25
½ ora dopo	2,39	2,50	45	0,11
1 » »	2,96	9,10	20	0,18
2 » »	3,07	5,—	75	0,37
3 » »	2,57	20,—	45	0,90
4 » »	—	3,60	45	0,16
Al mattino successivo	—	3,33	960	3,20
			1285	5,17

Prova della glicemia a digiuno senza somministraz. di HCl.

	Glicemia ‰	Glicosuria ‰	Quantità urine	Quant. tot. glucosio gr.
A digiuno	1,85	—	15	—
½ ora dopo	1,79	—	25	—
1 » »	1,70	—	25	—
2 » »	1,59	—	25	—
3 » »	1,55	—	30	—
4 » »	—	—	30	—
Al mattino successivo	—	10,—	900	9,—
			1050	9,—

Prova della glicemia a digiuno

previa somministrazione di cc. 50 di HCl al 0,5 %

	Glicemia ‰	Glicosuria ‰	Quantità urine	Quant. tot. glucosio gr.
A digiuno	2,32	—	50	—
½ ora dopo	2,14	—	30	—
1 » »	1,96	—	30	—
2 » »	1,78	—	40	—
3 » »	1,64	—	15	—
4 » »	—	—	45	—
Al mattino successivo	—	—	700	—
			910	—

Anche in questo caso risulta evidente l'azione ipoglicemizzante dell'acido cloridrico. La diminuzione del tasso glicemico si è resa più manifesta soltanto nella curva glicemica a digiuno (vedi fig. 4).

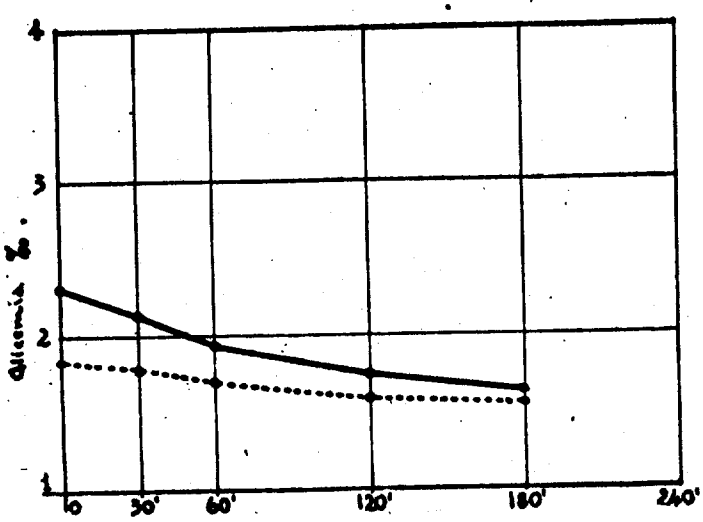

Fig. 4

Caso VII. — C. Cesare, a. 49, ferroviere, coniugato. *Diabete mellito.*

Di importante nell'anamnesi: ernia inguinale destra operata a 21 anni; blenorragia a 34 anni; da cinque anni ammalato col solito corteo sintomatico del diabete mellito. E' stato a più riprese sottoposto a trattamento insulinico.

E. O. — Pmx 130, Pmn 90 (R.R.). Quantità urine 2000; Ps 1030; Glucosio 62 ‰. Acetone ed acido acetacetico presenti. Nulla di particolare rilievo all'esame viscerale.

Curva della glicemia alimentare

(gr. 50 di gluc. in cc. 250 di acqua + cc. 50 di HCl al 0,5 %)

	Glicemia ‰	Glicosuria ‰	Quantità urine	Quant. tot. glucosio gr.
A digiuno	2,46	25,—	25	0,63
½ ora dopo	3,06	12,50	30	0,37
1 » »	3,14	16,50	20	0,33
2 » »	3,26	12,50	85	1,06
3 » »	2,71	12,30	100	1,23
4 » »	—	33,30	55	1,83
Al mattino successivo	—	30,—	2500	75,—
			2615	80,45

Prova della glicemia a digiuno senza somministras. di HCl

	Glicemia ‰	Glicosuria ‰	Quantità urine	Quant. tot. glucosio gr.
A digiuno	2,45	41,—	180	7,38
½ ora dopo	2,46	42,—	35	1,47
1 » »	2,39	33,—	30	0,99
2 » »	2,46	46,—	90	4,14
3 » »	2,32	25,—	50	1,25
4 » »	—	20,—	40	0,80
Al mattino successivo	—	62,—	2480	153,76
			2905	169,79

Prova della glicemia a digiuno previa somministrazione di cc. 50 di HCl al 0,5 %

	Glicemia ‰	Glicosuria ‰	Quantità urine	Quant. tot. glucosio gr.
A digiuno	2,64	25,—	200	5,—
½ ora dopo	2,44	25,—	20	0,50
1 » »	2,47	25,—	30	0,75
2 » »	2,52	14,—	25	0,35
3 » »	2,40	17,—	30	0,51
4 » »	—	14,—	15	0,21
Al mattino successivo	—	33,—	1600	52,80
			1920	60,12

In questo caso l'HCl non ha influito sul tasso glicemico anche nella prova della glicemia a digiuno.

Caso VIII. — M. Maria, a. 57, vedova. *Diabete mellito.*

Sofferente di diabete da 7 anni. Non ebbe mai a subire trattamento insulinico.

E. O. — Pmx 140, Pmn 70. Quantità urine cc. 2000; Glucosio 25 ‰. Negativo l'esame viscerale.

Curva della glicemia alimentare

(gr. 50 di glucosio + cc. 50 di HCl al 0,5 %)

	Glicemia ‰	Glicosuria ‰	Quantità urine	Quant. tot. glucosio gr.
A digiuno	2,15	3,75	115	0,43
½ ora dopo	2,65	1,—	35	0,035
1 » »	3,11	8,30	60	0,49
2 » »	3,08	16,60	25	0,41
3 » »	2,92	12,50	40	0,50
4 » »	—	14,—	20	0,28
Al mattino successivo	—	12,50	1750	21,87
			2045	24,015

Curva della glicemia a digiuno senza somministras. di HCl

	Glicemia ‰	Glicosuria ‰	Quantità urine	Quant. tot. glucosio gr.
A digiuno	2,10	2,7	55	0,14
½ ora dopo	2,10	1,5	10	0,015
1 » »	2,03	—	10	—
2 » »	1,88	—	45	—
3 » »	1,79	—	45	—
4 » »	—	—	20	—
Al mattino successivo	—	6,2	1500	9,30
			1685	9,455

Prova della glicemia a digiuno previa somministrazione di cc. 50 di HCl al 0,5 %

	Glicemia ‰	Glicosuria ‰	Quantità urine	Quant. tot. glucosio gr.
A digiuno	2,67	—	20	—
½ ora dopo	2,67	—	15	—
1 » »	2,45	—	30	—
2 » »	2,27	—	125	—
3 » »	2,14	—	90	—
4 » »	—	—	10	—
Al mattino successivo	—	2,50	1000	2,50
			1290	2,50

Pure in questo caso l'acido cloridrico ha esplicato la sua azione ipoglicemizzante sulla glicemia a digiuno; non ne ha risentito influenza la curva glicemica alimentare.

Prova della glicemia a digiuno nel non diabetico

Nome	Età	Malattia	Senza somministrazione di HCl Glicemia ‰ prima	30'	1 h	2 h	3 h	Previa somministrazione di cc. 50 di soluzione al 0,5 % di HCl Glicemia ‰ prima	30'	1 h	2 h	3 h
♀ S. L.	24	Epilessia	0,95	0,95	0,93	1,—	0,92	0,83	0,75	0,70	0,65	0,66
♀ S. C.	50	Esiti emorragici cer.	0,90	092	0,98	0,96	0,94	0,93	0,94	1,06	0,87	1,—
♀ L. G.	34	Esiti gonilite tbc. d.	0,90	0,92	0,89	0,94	0,90	0,89	0,85	0,88	0,87	0,90
♀ M. U.	40	M. di Basedow-Fl.	—	—	—	—	—	0,86	0,85	0,80	0,74	0,71
♀ P. I.	43	Nevrosi	—	—	—	—	—	0,95	0,86	0,91	0,83	0,88
♂ N. V.	51	Morbo di Addison .	0,90	0,88	0,92	0,86	0,88	0,98	0,93	1,06	0,95	1,—

Esperienze sull'uomo non diabetico

Riferisco senz'altro nella tabella i valori del tasso glicemico a digiuno prima e dopo la somministrazione per os di HCl in soggetti non diabetici.

Se ora diamo uno sguardo di assieme all'andamento delle curve glicemiche dei casi di diabete sopra riferiti, appare evidente come l'acido cloridrico, somministrato per os, abbia nettamente influenzato la glicemia.

La diminuzione del tasso glicemico si è verificata in modo pressochè uniforme, con intensità più o meno marcata, generalmente da un'ora a due ore dopo la somministrazione della soluzione cloridrica.

Non è però risultato un netto parallelismo tra il comportamento della curva glicemica alimentare e quello della curva glicemica a digiuno. Mentre in quest'ultima difatti l'HCl ha esplicato il proprio potere ipoglicemizzante (diminuzione del 28 %), l'aumento del tasso glicemico durante la glicemia alimentare non è stato invece frenato dall'HCl, salvo in qualche caso in cui sembra abbia favorito la discesa della glicemia che alla fine della terza ora raggiunse talvolta il valore di partenza.

Lo studio di controllo delle curve glicemiche a digiuno senza somministrazione di HCl ha dimostrato che nell'individuo diabetico è possibile durante il digiuno una diminuzione spontanea del tasso glicemico. Tale reperto già osservato da altri studiosi, pur dovendo essere tenuto presente nella valutazione dei reperti ottenuti colla somministrazione dell'HCl, non viene per altro ad infirmarli; modestissima è stata difatti la riduzione spontanea della glicemia a digiuno.

Nell'individuo non diabetico la glicemia è stata scarsamente influenzata dalla soluzione cloridrica; ciò corrisponde a quanto ebbero ad osservare Zunz e La Barre nelle ricerche di controllo sul cane normale ed è facilmente spiegato dalla ormai ben nota acquisizione che nell'individuo sano, anche tenuto lungamente a digiuno, il tasso glicemico subisce modestissime, spesso inapprezzabili variazioni. Anche Moretti, che si è a lungo occupato dello studio dei fattori neuro-ormonici nella patogenesi del diabete mellito, nelle sue ricerche di controllo su individui normali osservò costante insensibilità della glicemia a digiuno verso la ergotamina, alcaloide dotato come è noto di potere ipoglicemizzante.

I risultati delle osservazioni ora riferite confermano adunque le recenti ricerche sperimentali di Zunz e La Barre i quali nei cani surrenalectomizzati osservarono una costante diminuzione del tasso glicemico in seguito alla introduzione di HCl nel duodeno.

Secondo Gley e collaboratori tale fenomeno sarebbe legato ad ipersecrezione di insulina provocata dalla secretina. Infatti il Gley riscontrò potere ipoglicemizzante del siero di sangue proveniente dalla vena pancreatico-duodenale di cani cloralosati, nel cui duodeno era stata introdotta una soluzione di HCl. La ipoglicemia fu anche osservata in animali, la cui vena splenica era stata anastomizzata colla vena pancreatico-duodenale di altri animali datori trattati con iniezione intra-duodenale di soluzione cloridrica. Già per il solo fatto del passaggio del sangue venoso pancreatico di un animale nel circolo di un altro animale della stessa specie, il Gley e l'Hazard poterono osservare diminuzione del tasso glicemico, ma questa risultò ancor più marcata in seguito alla introduzione nel duodeno di HCl, che provocava abbondante secrezione pancreatica e formazione di secretina.

D'altra parte va ricordato che l'ipoglicemia consecutiva ad iniezioni di secretina non rappresenta puramente la conseguenza di una accentuazione della secrezione interna del pancreas. Infatti Zunz e La Barre hanno osservato nell'esperienza con la anastomosi pancreatico-giugulare che il tasso glicemico diminuiva gradualmente in misura più o meno marcata anche nel cane datore, il cui sangue venoso pancreatico veniva trasfuso in un altro animale. Inoltre il Takacs ottenne, sotto la influenza della secretina, una considerevole caduta della glicemia in cani spancreati.

In appoggio tuttavia della ipotesi che fa della iperinsulinemia provocata dalla secretina il principale fattore della ipoglicemia da acido cloridrico, parlano anche le esperienze di Dale, il quale avrebbe constatato nel cane aumento del tessuto insulare in seguito a ripetute iniezioni di secretina. Così pure Fieschi ha visto accrescersi, e talvolta perfino raddoppiarsi, la tolleranza per gli idrati di carbonio in animali (cane) sottoposti ad una determinata e costante razione ed ai quali era stata iniettata per un periodo di trenta giorni secretina preparata col metodo di Bayliss e Starling. Gli animali presentarono sempre per tutta la durata dell'esperienza lo stesso grado di ipoglicemia (da un'ora a un'ora e mezza dopo l'iniezione di secretina).

Ho già ricordato come Epstein e collaboratori abbiano dimostrato in vivo ed in vitro che la tripsina ostacola l'azione dell'insulina; tale fenomeno è stato attribuito alla formazione di un composto tra tripsina ed insulina.

Secondo Epstein i capillari sanguigni, pancreatici, in determinate condizioni, diverrebbero permeabili ai prodotti della secrezione degli acini e specialmente alla tripsina o meglio alla protripsina o tripsinogeno. Seguirebbe così neutralizzazione, od immobilizzazione come ritiene il Grott, dell'insulina, ed ancora per il passaggio di protripsina nella circolazione portale aumento della glicogenolisi epatica, con iperglicemia ed anche glicosuria. Questo, secondo l'Epstein, sarebbe il meccanismo col quale la tripsina contribuirebbe alla regolazione della secrezione interna pancreatica. Quando gli isolotti secernono in eccesso la insulina, che come sappiamo è dotata di potere succagogo, il passaggio di tripsina (o di tripsinogeno) nel sangue proveniente dagli isolotti, contribuirebbe ad ostacolare gli effetti dannosi della ipoglicemia. Inoltre l'iniezione di insulina nella vena pancreatico-duodenale, invece di provocare ipoglicemia, determinerebbe nel gatto, sempre secondo l'Epstein, iperglicemia e glicosuria per il passaggio di protripsina nel sangue venoso degli acidi. Al contrario l'introduzione nell'arteria pancreatico-duodenale di insulina mescolata a soluzione di saffranina (sostanza colorante che intimamente si fissa alle granulazioni zimogene degli acini) non determinerebbe nè iperglicemia nè glicosuria. L'Epstein per interpretare tale fenomeno ha prospettato l'ipotesi che la combinazione fra protripsina e sostanza colorante sia molto stabile e che perciò risulta ostacolato il passaggio del tripsinogeno del sangue venoso degli isolotti, donde la mancata neutralizzazione della insulina.

Seguendo le vedute di Epstein si sarebbe indotti a ritenere che, diminuendo la quantità di protripsina contenuta negli acini, l'insulina possa esplicare più intensamente la sua azione nell'organismo. Potremmo quindi supporre che quando si provoca la secrezione pancreatica esterna sia con HCl o con secretina, diminuisca la quantità di protripsina presente nel pancreas per il passaggio di essa nell'intestino e nel contempo la immissione in circolo di una maggiore quantità di insulina, e perciò una diminuzione del tasso glicemico.

Secondo il Dixon e l'Hamill, come pure secondo l'Hustin, la secretina verrebbe facilmente fissata dagli elementi secernenti degli acini ghiandolari. In base a questo reperto, si potrebbe avanzare l'ipotesi che la azione ipoglicemizzante della secretina di cui è stata eccitata la formazione con la soluzione cloridrica, dipenda dalla diminuzione della tripsina, che ha sicuramente, almeno in vitro, potere neutralizzante sulla insulina. Va però ricordato che varie obiezioni sono state mosse alla teoria di Dixon ed Hamill e di Hustin sulla secrezione esterna del pancreas, come pure a quella di Epstein riguardante la influenza della tripsina sulla glicoregolazione. La spiegazione pertanto del meccanismo d'azione della secretina sulla glicemia rimane per ora, come ebbero a scrivere Zunz e La Barre, puramente ipotetica, tanto più che il Takacs avrebbe osservato potere ipoglicemizzante della secretina anche in animali spancreatizzati.

Ma pur tralasciando la questione del modo di agire della secretina sulla secrezione pancreatica interna, ci si presenta il problema riguardante il meccanismo per il quale la secrezione pancreatica esterna si accompagna dopo introduzione di HCl a diminuzione del tasso glicemico. Non siamo in alcun modo autorizzati a concludere che tale fenomeno sia puramente legato a formazione di secretina. Meyer, Bisch, Bock e Wohlenberg, come pure Martino credono di avere isolato nel secreto pancreatico esterno dell'uomo e del cane un principio iperglicemizzante, antiinsulinico; la iniezione di ultrafiltrato di succo pancreatico oppure la somministrazione per os del succo pancreatico stesso provocherebbe, secondo questi AA., aumento del tasso glicemico tanto nei conigli quanto nel cane.

Di fronte a tali reperti la diminuzione del tasso glicemico, come ebbero già a prospettare Zunz e La Barre, potrebbe anche dipendere dall'azione antagonista del principio iperglicemizzante verso l'insulina, sia nel pancreas che nel sangue circolante. Solo ulteriori indagini potranno chiarire questo quesito; resta ad ogni modo dimostrato il potere ipoglicemizzante dell'HCl sul quale richiamò pure l'attenzione l'Usuelli, che, nel 1927, ebbe a prospettare la possibilità che l'iperemia del pancreas, consecutiva ad iniezione di secretina o ad introduzione di HCl nel duodeno, oltre che influire sulla secrezione esterna, fosse in grado di determinare iperattività dei distretti insulari.

Nel diabetico adunque la somministrazione dell'HCl esplica generalmente benefica influenza, sebbene temporanea, sulla glicemia. Tale rilievo ha naturalmente modesta portata pratica; non mi sembra tuttavia privo di interesse dal punto di vista teorico. Esso ci richiama allo studio della secrezione gastrica e della secrezione pancreatica esterna del diabetico. Per quanto riguarda la secrezione gastrica nei diabetici non molto numerosi sono infatti gli studi. Da questi sembrerebbe risultare che frequentemente il diabetico soffre di ipo-anacloridria (25 % secondo lo Joslin, 32 % secondo il Wiechmann e l'Elzas, l'89 % secondo recenti studi di Rabinovich, Fowler e Vatson).

Anche la secrezione pancreatica esterna risulterebbe diminuita nel diabetico come avrebbero osservato Chiray, Salmon, Mercier, Gavrila e Paraschivesco ed altri studiosi.

Pure tralasciando tali quesiti che potranno essere chiariti soltanto da ricerche sistematiche, un fatto emerge da quanto sono venuto esponendo: la conferma cioè della esistenza di rapporti tra secrezione gastrica e funzione pancreatica interna.

Donde la necessità di tenere presente nella patogenesi del diabete mellito accanto all'elemento fondamentale, « insufficienza pancreatica », anche il fattore « insufficienza gastrica ».

Riassunto. — La somministrazione per os di acido cloridrico determina nei diabetici una diminuzione del tasso glicemico più o meno marcata (20 - 30 %) in modo pressochè uniforme.

Il potere ipoglicemizzante dell'HCl è specialmente manifesto a digiuno.

Nei soggetti che non presentano disturbi della sfera digerente la glicemia è solo scarsamente influenzata dalla somministrazione di acido cloridrico.

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Brummer-'48
Bib no. 47

Acta Med. Scand. 130(6):559-574. 1948.
Acta Medica Scandinavica. Vol. CXXX, fasc. VI, 1948.

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On the Effect of Some Common Gastric Drugs on the Motility of the Stomach.

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(Submitted for publication September 18, 1947.)

As previously reported by Brummer and Wegelius, motility disturbances of the stomach can be established by X-ray with the majority of persons suffering from gastric distress, whereas most healthy persons are not found to have such disturbances. Accordingly a definitely more distinct relation can be indicated between gastric distress and motility disturbances than between gastric distress and chronic gastritis, not to speak of secretory disturbances. Brummer and Wegelius therefore assume motility disturbances of the stomach to be the probable direct cause of gastric distress; the diagnosis would thus be gastric dystonia and not dyspepsia. The type of motility disturbance is evidently of less significance in this connection than the tendency to such disturbances, for, as already indicated by Weltz and as clearly revealed by the investigations described in the present paper, the type of motility disturbance established may vary from time to time. For instance, one examination may disclose with the same patient a greatly increased peristalsis and another examination a total absence of peristalsis.

It is the object of the following paper to endeavour to find additional corroboration for the hypothesis presented on the significance of motility disturbances as cause of gastric distress by describing X-ray observations of the effect of various gastric drugs on the motility of the stomach, for in case motility disturbances truly are the cause of gastric distress it can be expected that drugs

which give relief to the symptoms of distress will be found to affect gastric motility when the question is not of a purely narcotic effect. It is not proposed to deal in the present connection with the effect of different drugs on gastric motility in the full extent of the question but merely to endeavour to study the effect in this respect of a few of the most common gastric drugs when administered per os in the usual therapeutic doses.

Of the drugs investigated, the action of atropine and papaverine has already in the past been considered to be due to their anti-spasmodic effect, in other words to direct action on gastric motility. On the other hand, the relief obtained in gastric distress from various antacids is generally ascribed to their power to neutralize hydrochloric acid. Hydrochloric acid, again, has been regarded as a substitute for absence of gastric acid regardless of the fact that even a dose of 4 ml diluted hydrochloric acid taken with meals has no effect worth mentioning on gastric acidity (Brummer).

The drugs under observation in the present study in regard to their effect on gastric motility are atropine, sodium barbital as representative of the barbiturates, papaverine, hydrochloric acid, and various antacids. The method followed in the observation of the first three drugs comprised a number of X-ray examinations on separate days. Before one of these examinations — the control examination — no drug was administered; one half to one hour before all the other examinations the patient received a dose of the drug then under observation. A different order of examination was followed for each patient, and the X-ray investigator (Bundul) had no knowledge of the drug used in the specific case being examined. With the doses of hydrochloric acid and antacids, the usual X-ray examination was first performed, followed by administration of the drug and observation of possible resultant variations in gastric motility. Attention was paid to peristalsis and tone of the stomach and the duodenum and to any deviations from the normal in them. As was already mentioned, considerable variation in the type of motility disturbance could often be observed in the same patient at the different examinations, and for this reason importance cannot be given to the results of individual examinations but only to the combined result of several examinations. The drugs observed were, as stated, administered per os.

All the patients observed were hospitalized for treatment in the medical department of the Provincial Hospital at Oulu. The material consists mainly of patients complaining of the usual.

so-called dyspeptic distress; however, in view of what has been stated earlier in this paper, the term dyspepsia will be herein substituted by the diagnosis gastric dystonia. Some cases of peptic ulcer and gastric carcinoma and a few healthy persons are also contained in the material. A total of 137 persons were studied, with 316 X-ray examinations performed. A closer description of the material in each instance is obtained from the account on the results obtained with the several drugs. In addition to the X-ray examination, the usual gastric analysis was carried out in the case of a part of the patients in the manner more fully described in an earlier paper by Brummer on gastric motility disturbances.

Atropine.

It is generally regarded that atropine has an inhibitory effect on the motility of the stomach by reducing both peristalsis and tone. Its effect lies mainly in a paralyzing action on the vagus; Barsony and v. Friedrich have found it to also directly affect the stomach muscles. In animal experiments in particular, the inhibitory action of atropine on peristalsis and tone is clearly evident when the drug is administered in sufficiently large doses (*e. g.* Zuntz and Tyrebaert, Smith). According to Lockwood and Chamberlin, on the other hand, the human stomach is considerably less sensitive to atropine than that of animals, and for this reason they hold that only the maximum doses are sufficient to shorten the emptying time. Inhibition of gastric motility in man with doses ranging from 0.5 to 1.5 mg is established by i. a. Östvös, Lasch, Petrovic and Tetelbaum. Östvös also finds that atropine in doses of 0.5 to 1.0 mg may, in addition to inhibitory effect, also cause pylorospasm, as had already been indicated by Wertheimer and Boulet with large doses. A relatively negative attitude toward peroral atropine therapy is taken by Bastedo, who is of the opinion that the doses required to produce an effect on gastric spasms are so large as to produce unpleasant by-effects.

In our own material the effect of atropine was observed with 40 patients. Thirty-three complained of gastric dystonia distress, a peptic ulcer was established with two and a gastric carcinoma with 3 patients, one had a disease of the bile duct, and no gastric symptoms were established with two.

The dose was 0.5 mg atropine sulphate, administered per os one-half to one hour before X-ray examination.

In the control material peristalsis was normal with 11 patients and tone with 25; increased peristalsis was found with 12 and increased tone with 5 patients; with 17 and 10 patients respectively they were decreased.

Following the administration of the atropine, peristalsis was normal with 5 and tone with 20 patients, both peristalsis and tone were increased with 8, and peristalsis was decreased with 27 and tone with 12 patients. These results already indicate that the dose of atropine administered apparently decreases the peristalsis to some extent, whereas no effect can be observed in regard to the tone. The effect on the peristalsis can be seen still more clearly by observing the change after atropine in each individual case as compared with the control examination. It was then found that the peristalsis was unchanged with 9, increased with 7, and decreased with 24 patients. For tone the corresponding figures were 16, 11 and 13.

On the basis of these results it can be stated, corroborating earlier finds reported in the literature, that a dose of 0.5 mg of atropine administered per os apparently has a decreasing action on gastric peristalsis; on the other hand, no effect on the tone could be established. The decreasing action of atropine on the peristalsis is not, however, so strong — at least not at the doses used in these cases — as to bring out the action in every instance. In those cases where peristalsis appeared to have increased after atropine, this probably is not accountable to the drug, a more natural explanation probably being that in these cases atropine was not able to inhibit spontaneous variations in a pathologically sensitive gastric peristalsis.

Sodium Barbital.

We have found in literature no detailed information on the effect of barbiturates on gastric motility.

The investigations were carried out with the same material as those on atropine, and each patient was accordingly subjected to X-ray examination three times. After inclusion of one person with no gastric symptoms the material totalled 41 cases.

Prior to the X-ray examination 0.1 g of sodium barbital was given to the patient. The examination disclosed that the peristalsis, in comparison with the findings in the control examination,

was unchanged with 10, increased with 22, and decreased with 9 patients. For the tone the figures were 18, 12 and 11 respectively. As was the case also with atropine, no effect on the tone could be established with the administered dose of sodium barbital, but there apparently was a slight increase in the peristalsis. This effect, which at first may seem somewhat surprising, is apparently due to barbiturates having — like all thalamic narcotics in general — a stronger sympathetic than parasympathetic inhibitory effect, the result being a relative increase in the vagus tone, in a similar manner as in physiologic sleep. As is known, barbiturate poisonings bring on increased gastro-intestinal motility, such as a tendency to vomiting and diarrhea.

Papaverine.

Gross and Slaughter have found with animal experimentation that 2 to 6 mg of papaverine per kilogramme administered intravenously or intramuscularly reduces the peristalsis and tone in the stomach but not in the small intestine. The same observation was made already earlier by i. a. Plant and Miller. On the other hand, Barsony and v. Friedrich were unable to find any effect on the human gastric tone and peristalsis or on the opening and closing of the pylorus with intravenous doses of 0.01—0.08 g or intramuscular doses of 0.12 g. Information on the action of papaverine on pylorospasms is somewhat contradictory. According to some investigators papaverine can always or nearly always reduce these spasms, whereas other investigators consider its effect uncertain in most cases (Holzknecht and Sgalitzer, Szerb and Révész, Schlesinger, Erdélyi).

In the present material the effect of papaverine was observed with 36 patients, of which 29 belonged to the gastric dystonia group, 3 complained of no gastric distress, 2 were patients with peptic ulcer, and 2 with gastric carcinoma.

A dose of 0.05 g of papaverine hydrochloride was given per os to 25 of these patients prior to the X-ray examination. A comparison with the control examination disclosed that the peristalsis was unchanged with 6, increased with 10, and decreased with 9 patients. For the tone the figures were 18, 3 and 4 respectively. According to these results the administered dose of 0.05 g had no effect on the gastric peristalsis and tone, nor could any change be observed in the duodenal motility.

In view of this negative find the remaining 11 cases were given a larger dose of papaverine, consisting of 0.1 g administered likewise per os one-half to one hour before the X-ray examination. In several of these patients lassitude was clearly observable after taking the drug.

Even with this dose no effect on the gastric peristalsis and tone could be established. For instance the peristalsis was unchanged with 3, increased with 4, and decreased also with 4 patients. However, an effect was found on the duodenal motility. The peristalsis and tone of particularly the bulbus duodeni decreased distinctly. The bulbus either filled poorly or filled atonically, whereafter the contrast medium ceased to move; when the bulbus was pressed to empty it, the contents generally passed back into the stomach. Changes of this kind in the motility of the duodenum were established with 10 patients out of the 11 examined; with 2 patients also duodenal retroperistalsis was observed.

Corroborating the statements made in the literature, the present investigations disclose no effect on gastric motility with the administered doses of papaverine, but the dose of 0.1 g apparently had action on the duodenal motility.

Hydrochloric Acid.

In 1907 Cannon presented his well known theory on acid control of the pylorus, according to which increased gastric acidity causes the opening and increased duodenal acidity the closure of the pylorus. This opinion, on the basis of which the theory of rapid emptying of an achlorhydric stomach was developed, has in the light of modern research proved erroneous, for there is no separate contraction of the pylorus, it being a part of the peristalsis of the stomach. According to several investigators (*e. g.* McClure et al., Baird et al., McCann) the acidity of the contents of the stomach and duodenum has no effect on the emptying time of the stomach. Brummer, using methylene blue as indicator, could also establish no difference in the emptying time whether the achlorhydria patient took water or hydrochloric acid on an empty stomach.

In the present investigations the effect of hydrochloric acid on gastric and duodenal motility was observed with 13 patients, with 4 of whom insulin-proved achlorhydria was established; among the latter were 2 patients suffering from addisonian per-

nicious anaemia. The usual gastric X-ray examination was first performed, after which the patients received 1 ml of diluted hydrochloric acid in 1 dl of water.

Changes in the gastric tone were not established with patients. With 5 patients, one of whom suffered from achlorhydria, no effect could be found even on the gastric peristalsis; with 4 patients, two of whom had achlorhydria, the peristalsis was slightly decreased after administration of the drug but in one of these it again increased after 10 minutes; with the remaining 4 patients, 1 of whom suffered from achlorhydria, a very slight increase of the peristalsis was found. To summarize it therefore can be stated it could not be established that the administered dose of hydrochloric acid has any distinct effect on the gastric motility.

The duodenal motility and in particular its retroperistalsis was found to increase during the examination of 6 cases. After administration of the hydrochloric acid the contrast medium at first moved backward and forward in the duodenum, returning partly to the stomach, and only after some time was able to pass onward to the intestine. It may be mentioned that retroperistalsis which was as clearly distinguishable could be established in the entire material only once in the control examination. Two of the 6 patients with whom hydrochloric acid was found to act on the duodenal peristalsis suffered from achlorhydria.

Summarizing these results it can be stated that the administered dose of 1 ml of diluted hydrochloric acid has no distinct effect on gastric motility, but in a part of the cases it caused increased peristalsis and particularly retroperistalsis of the duodenum, which seemed to be independent of whether the patient had acid gastric secretion or not.

Antacids.

Investigations were performed with the following antacids: Sodium bicarbonate, calcium carbonate, magnesium carbonate, magnesium oxide and »Ventracon» prepared by Orion Pharmaceutical Factory and containing per 100 g : 12 g aluminium hydroxide, 5 g calcium phosphate, 23 g magnesium peroxide (25 %) and 60 g bolus alba. In accordance with information previously given in the literature it is generally claimed that sodium bicarbonate increases and magnesium compounds decrease the gastric motility (Meyer and Gottlieb: Die exp. Pharmakologie).

In the investigations the following doses were administered to the patients: ca. 7 g sodium bicarbonate, ca. 5 g calcium carbonate, ca. 2 g magnesium oxide, and ca. 6 g Ventracon. With the exception of Ventracon the neutralizing property of these amounts is approximately equivalent, ranging from 70 to 90 ml of 1 NHCl, whereas 6 g Ventracon can only neutralize from 35 to 40 ml of 1 NHCl. That Ventracon was administered in doses only half as large as the others with respect to its neutralizing property was due to the fact that an unsuitably large volume would have been required to achieve with it the same neutralizing effect as with the other substances.

The results obtained were as follows:

1) Sodium Bicarbonate.

The material consisted of 10 patients with gastric dystonia. Four of them were found to have insulin-proved achlorhydria; 2 of the latter suffered from addisonian pernicious anaemia. Further there were 2 patients with peptic ulcer and one with gastric carcinoma.

The peristalsis was observed to increase after the administration of sodium bicarbonate in all of the 10 patients first mentioned. The increase was very distinct with 9 patients and less pronounced with one. As a rule ten minutes subsequent to the patients' receiving the dose a deep and large wave of peristalsis was visible moving through the stomach. In two of the cases also the tone seemed to increase. The effect of the sodium bicarbonate was as distinct with the patients with achlorhydria as with the others. No definite effect was observed with one of the peptic ulcer patients and the carcinoma patient.

2) Calcium Carbonate.

Six patients with gastric dystonia and one with peptic ulcer were examined. Two of the former were found to have insulin-proved achlorhydria.

The calcium carbonate had no effect on the gastric peristalsis or tone of 3 of the patients among whom were both the achlorhydria cases. With 4 patients a slight increase of peristalsis was observed. Thus calcium carbonate possibly has a slightly increasing effect on gastric peristalsis, which is not however manifested by an achlorhydric stomach evidently due to the insolubility of the calcium carbonate.

3) Magnesium Carbonate.

The effect of magnesium carbonate was observed with 14 patients. Peptic ulcer was established with 2 of them, while the remaining 12 belonged to the gastric dystonia group. Insulin-proved achlorhydria was present in 3 of the latter.

Gastric peristalsis increased subsequent to administration of the magnesium carbonate distinctly with 3 patients and less distinctly with 8 of them, whereas no effect could be observed with 3, among whom were 2 patients with achlorhydria. With respect to tone no effect was observed. Very typical of magnesium carbonate was its effect on the duodenal peristalsis, which increased with 8 patients, with 7 of whom distinct retroperistalsis was also manifested. Taking into consideration the effect of this drug both on the gastric and the duodenal peristalsis, the material includes only 2 cases where no effect was established. Since both of these were patients with achlorhydria it appears that the effect of this drug, which is not easily soluble, is dependent on the presence of hydrochloric acid, which also was the case with calcium carbonate.

4) Magnesium Oxide.

The material comprised 3 patients with peptic ulcer, 2 with no gastric symptoms, and 11 with gastric dystonia. Insulin-proved achlorhydria was present with 3 patients.

This drug was not found to have any effect on the gastric motility with 4 of the patients, 2 of whom had achlorhydria, whereas it was observed that the gastric peristalsis increased slightly with 10 and distinctly with 3 patients. No effect on the gastric tone was established. Like magnesium carbonate, magnesium oxide also increased the duodenal peristalsis. With 9 of the 17 patients examined it was observed that the duodenal peristalsis increased after administration of magnesium oxide; retroperistalsis was in evidence with only one patient. No effect was noted with 2 of the achlorhydria patients and even with the third the increase of gastric peristalsis was hardly evident.

The effects of magnesium oxide and magnesium carbonate resemble each other in many respects, the former, however, being evidently somewhat slighter than the latter. The effect of magnesium oxide on patients with achlorhydria, like that of other antacids not easily soluble, was less evident than on patients with acid gastric secretion. The effect of the magnesium salts on

duodenal peristalsis must doubtless be collorated with their promoting effect on bile secretion.

5) Ventracon.

Twelve patients were examined. Two of them had no gastric symptoms, and the rest suffered from gastric dystonia. Achlorhydria was established with 4 of them.

A distinct increase of gastric peristalsis was observed with only one of the achlorhydria patients. No effect was perceived with 2, one of whom had achlorhydria. With the remaining 9 patients gastric peristalsis increased only slightly after administration of Ventracon. An effect on the duodenal peristalsis was seen only with 2 patients. Thus the effect of Ventracon on gastric and duodenal peristalsis seems with most patients to be slight and evidently independent of gastric acidity.

Discussion.

In summarizing the observations presented above it may be stated that most of the drugs under observation have, when administered in the usual therapeutic doses, a more or less distinct effect either on gastric or duodenal motility or on both. Atropine decreases gastric peristalsis, whereas sodium barbital increases it. Sodium bicarbonate has a distinctly increasing effect on gastric peristalsis, which is also true of the other antacids employed, though the effect is considerably slighter. The magnesium salts have also an effect on the motility of the duodenum. Likewise hydrochloric acid had an effect on the motility of the duodenum, but no distinct effect on the motility of the stomach was to be observed. Only papaverine in a 0.05 g dose failed to have any effect perceivable, but when the dose was increased to 0.1 g it decreased the motility of the duodenum and particularly that of the bulbus duodeni, whereas it had no observable effect on the stomach.

The fact that the drugs which relieve gastric distress generally have an effect on gastric or duodenal motility gives rise to the idea that their therapeutic effect may at least partly be due to the latter effect. In the following this question is dealt with in detail with reference to the several drugs in the light of the results presented above and of some additional experiments.

It is already earlier generally regarded that the effect of atro-

pine on gastric distress is based at least partly on its antispasmodic property. Tetelbaum and others have stated that, at the same time as the gastric peristalsis is inhibited by atropine, the patient's distress is relieved. The inhibitory effect of atropine on gastric peristalsis was also observed in the authors' investigations. Furthermore it has been claimed that atropine prevents gastric secretion and consequently relieves hypothetic distress caused by hypersecretion. However, the authors have found with gastric patients, some of whom had achlorhydria and some acid gastric secretion, that atropine relieves gastric distress with equal success in both groups, wherefore it is evident that its therapeutic effect may not be based on its inhibitory action on gastric secretion.

The use of hypnotics, particularly the barbiturates, in gastric distress, especially nausea, has been accounted for because of their sedative effect on the stomach. The opinion may also be held that particularly in nausea, where the stomach is frequently roentgenologically entirely atonic at least at the initial phase, the peristalsis-increasing effect of the barbiturates, which was established above, promotes evacuation and consequently relieves distress.

The effect of papaverine has also previously been considered to be due to its antispasmodic properties. It is not known to have any effect on secretion. But, since the 0.05 g dose, which according to tests made by authors also relieves gastric distress in many cases, has no effect on the motility of the stomach, as it was established above, it seems more natural to conclude that its effect is for the great part purely narcotic.

Hydrochloric acid has been one of the most popular and uncritically used gastric drugs for the last fifty years. It has been believed for instance to promote peptic digestion and consequently relieve gastric distress. As Brummer has demonstrated, the therapeutic results claimed to have been obtained by it have at least in part been of a psychotherapeutical nature. For, as it has already been mentioned in an earlier paper, even 4 ml of diluted hydrochloric acid taken during a meal has no noteworthy effect on the acidity of the gastric contents and consequently has no promoting effect on peptic digestion. This dose can only make the contents of an empty stomach slightly acid for some time. Admittedly, there are cases in which hydrochloric acid has a favorable effect on gastric distress without any psychotherapeutic factors. Thus, in the therapeutic experiments with antacids

described below it was found with a female patient that neither sodium bicarbonate nor magnesium oxide relieved her gastric distress, whereas hydrochloric acid did. The patient had never used the latter before, wherefore she was not familiar with its taste. It was roentgenologically verified that hydrochloric acid had a distinct effect on her duodenal peristalsis, and all gastric analyses revealed normal acidity. It appears that the relieving effect of hydrochloric acid — when it is actually verified — can more easily be accounted for on the grounds of its effect on motility than for instance of a promoting effect on chemical digestion particularly when, as in the above case, the therapeutic as well as the motility effects of hydrochloric acid are not necessarily connected with gastric acidity.

The general conception is that the effect of the antacids is due to their neutralizing property. Furthermore, it is mentioned in the literature that in using antacids containing carbonates, carbonic acid is released in the stomach through the effect of hydrochloric acid and it is supposed to have sedative effect on the stomach. In this connection attention has not been drawn to the peristalsis-increasing effect of sodium bicarbonate, which has been known for a long time.

If the relieving effect of antacids on gastric distress were based on their neutralizing property, first, the latter should be dependent on gastric acidity, and, second, one would expect the therapeutic effect of the several antacids to be at least in some proportion to their neutralizing property. The first condition does not hold good, for as among others Brummer has stated, sodium bicarbonate more frequently gives relief in the gastric distress of patients with achlorhydria than hydrochloric acid. With the purpose of going into this question more thoroughly and comparing the relief to gastric distress given by different antacids, therapeutic experiments were performed with natrium bicarbonate, Ventracon and magnesium oxide. Patients were told that they will be given two drugs and were requested to state which of them gave greater relief to their gastric distress. The doses administered were: 1 g sodium bicarbonate and 2 g Ventracon (the neutralizing property of each is from 10 to 15 ml 1 NHCl) and 1 g magnesium oxide, the neutralizing property of which is greater than that of the first mentioned drugs (ca. 35 ml 1 NHCl).

A comparison was made between the therapeutic effects of sodium bicarbonate and Ventracon with 38 patients, 3 of whom

suffered from peptic ulcer and the remaining 35 from gastric dystonia. Insulin-proved achlorhydria was present with 12 of the latter group. Twenty-one of the patients considered sodium bicarbonate more relieving and 10 Ventracon. Of the first group 8 had achlorhydria and of the second 4. Of the 7 remaining patients 4 felt equally relieved by both the drugs, and 3 felt that neither one have any relief.

A comparison was also made between the therapeutic effects of sodium bicarbonate and magnesium oxide on 21 patients with gastric dystonia; 6 of them were found to have achlorhydria. Eighteen, among them all the achlorhydria patients, preferred sodium bicarbonate. The remaining 3 considered both equally good.

The results confirm the observation that the relieving effect of sodium bicarbonate on gastric distress is independent of the gastric acidity. Also Ventracon seems to relieve the distress of achlorhydria patients. As it was stated above, the effect of both the drugs on also the motility of the stomach was independent of gastric acidity. Further, the experiments show that sodium bicarbonate, which has roentgenologically the most distinct effect on the motility of the stomach, is also considered by most of the patients to give the best relief in their gastric distress, regardless of the fact that the neutralizing property of the dose of Ventracon was equal to and that of the dose of magnesium oxide from two to three times as great as that of the sodium bicarbonate dose.

The authors draw the conclusion from the experiments that it is more probable that the relieving effect on gastric distress of the different antacids is due to their effect on the gastric motility than to their neutralizing property.

To summarize, the observation may be made that the drugs experimented generally affect the gastric motility more or less distinctly even when administered in only the usual therapeutic doses, and that it is highly probable that their relieving effect on gastric distress is at least partly due to the said effect on motility, which confirms the hypothesis presented in the beginning with reference to the significance of motility disturbance in the etiology of gastric distress.

The hypothesis that an explanation for the effect of gastric drugs is at least partly to be found in their effect on the motility of the stomach opens new vistas to attaining causal medical therapy in gastric distress. Perhaps by following this conception we

can in the future find a better explanation than hitherto for the therapeutical results that at present seem so arbitrary — that is, find an answer to why sodium bicarbonate has a better effect on one patient and Ventracon on another, and perhaps hydrochloric acid on a third one, and why atropine is more suitable for one patient and papaverine for another, etc. Meanwhile we can only ascertain that the evident cause, at least for a considerable part, of gastric distress is disturbances in the motility of the stomach, wherefore different drugs, each of which affects the gastric motility in its own typical way, give relief. That the suitability of a drug varies with different cases depends upon factors as yet unknown, such as perhaps the type and degree of the disturbance in motility, the reactions of the organism of different individuals, etc. However, it seems possible that by continuing experiments on the above explained foundation we shall with greater probability be able to find the laws governing the said therapeutic effect than in the light of, for instance, disturbances in gastric secretion.

Summary.

The authors have performed X-ray examinations on the effect of the usual gastric drugs on the motility of the stomach. The drugs used were atropine, sodium barbital, papaverine, hydrochloric acid, and five different antacids, viz. sodium bicarbonate, calcium carbonate, magnesium carbonate, magnesium oxide, and »Ventracon» (Orion's preparate containing per 100 grammes, according to manufacturer's statement, 12.0 g aluminum hydroxide, 5.0 g calcium phosphate, 23.0 g magnesium peroxide (25 per cent) and 60 g bolus alba). The drugs were administered per os, generally in the usual therapeutic doses. 137 patients underwent observation, and the number of X-ray examinations totalled 316.

It was established that atropine decreased and sodium barbital increased the gastric peristalsis. After a dose of 0.05 g of papaverine no change could be observed, whereas 0.1 g of papaverine had a decreasing effect on the peristalsis and the tone of the duodenum and of the bulbus duodeni in particular; on the gastric motility even this dose was found to have no effect. Hydrochloric acid, likewise, had no distinct effect on the gastric motility, while it increased the duodenal peristalsis, causing in particular retroperi-

stalsis. Among the antacids, sodium bicarbonate very clearly increased the gastric peristalsis, which was also true, although in a less marked degree, of the other antacids used; the magnesium salts furthermore acted on the duodenal peristalsis.

It is the opinion of the authors that the benefit derived by patients from the drugs under observation is due to their effect on gastric and duodenal motility. This opinion was confirmed by certain comparative therapeutic experiments carried out with antacids, in which it was established that sodium bicarbonate, which had the most distinct effect on the gastric motility, relieved the gastric distress of the patients distinctly better than magnesium oxide or Ventracon, regardless of the fact that the neutralizing properties of the administered Ventracon dose were as great, and those of the magnesium oxide dose two or three times as great as those of the sodium bicarbonate. Also it appeared that the therapeutic effect of sodium bicarbonate and Ventracon as also the effect of these antacids on the gastric motility was independent of the patient having achlorhydria or an acid gastric secretion.

The authors state in conclusion that although it accordingly appears probable that the relief obtained in gastric distress from various drugs is due at least in part to their effect on gastric motility, the details of this effect are still unsolved and it therefore is not known why one patient obtains more relief from sodium bicarbonate and another patient e. g. from Ventracon, while others may find atropine, papaverine or some other drug more suitable. It merely is evident that the motility disturbances which underlie gastric distress are in one case affected more beneficially by a different drug than in another case. However, it is possible that continued research based on the finds described above may shed more light on the question and increase our knowledge of the causal therapy of gastric distress.

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Burns-'29
Bib No. 52

Biochem. Jour. 23(5):860-867. 1929.

XCIV. THE EFFECT OF THE CONTINUED INGESTION OF MINERAL ACID ON GROWTH OF BODY AND BONE AND ON THE COMPOSITION OF BONE AND OF THE SOFT TISSUES.

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(*Received July 2nd, 1929.*)

Strong acid introduced into the animal body appears to be mainly excreted in combination with ammonia, but evidence exists to show that some disturbance of the relative proportion of the fixed bases in the blood is produced [Stewart and Haldane, 1924; Allers and Bondi, 1907], and that some extra excretion of the fixed base of the body may be brought about [Steenbock, Nelson and Hart, 1914; Givens and Mendel, 1917; Stehle, 1917; Lamb and Evvard, 1919; Shohl and Sato, 1923; Hunter and Aub, 1927]. The nature of the extra fixed base thus excreted would seem to be determined by the excess of available base, which depends on the diet [Lamb and Evvard, 1919; Stehle and McCarty, 1921].

Gamble and Ross [1923] concluded that most of the extra base excreted after the administration of acid was merely washed out in combination with the usual acids of the body as a result of the diuresis which follows such administration, but Fiske, Goodell, Hathaway and West [1926] found that fixed base was excreted in excess of the base merely washed out in this way. After this preliminary loss of base, however, they noted a subsequent retention. All these conclusions were drawn from short metabolism experiments. Goto [1918] examined rabbits to which acid had been administered for various periods. He found a great loss of weight, a loss of fat and carbonate, but not of calcium of bone and a reduction in the average potassium and phosphate content of the muscles of the acid group as compared with the controls. The latter varied greatly among themselves, however, and the two groups overlapped considerably. The rabbits were adults of varying age and breed.

Lamb and Evvard [1919] examined the bones of a pig to which they had administered acid for a long period. The amount of marrow was much reduced. No chemical examination was made.

Experimental.

The following experiment was carried out to determine whet acid ingestion exerted any influence on the storage of base in the tissues oung growing rabbits and rats. The animals used for the experiment were bred in the laboratory from stock kept on standard diets, and were reared under carefully controlled conditions; animals which received acid were only compared with litter-mate controls. It was hoped in this way to detect small changes in bone and tissue composition.

Their age at the beginning of the experiments was about 6 weeks, but such great difficulty was experienced in rearing more than one or two rabbits in each litter, that the later experiments were carried out on rats.

Diet. The diet of the rabbits consisted of oats, and bran mash with lettuce or cabbage *ad libitum*.

The rats received Steenbock's stock diet [Bethke, Steenbock and Nelson, 1923], with fresh milk and water *ad libitum*.

Administration of acid. The acid used was 0·1 *N* hydrochloric acid. In the first experiment, carried out on two rabbits, with one litter-mate control, the acid was given by stomach tube, but this caused acute local lesions in the stomach and one rabbit had to be discarded. At the end of 17 days the second animal died. The bones of this animal and of its control were taken for chemical examination.

In all later experiments, the acid was administered by mixing it with the mash in the case of the rabbits, and with the maize mixture in the case of the rats. Under these circumstances it was not possible to know accurately the amount of acid consumed, as some scattering of food always took place, but an approximate estimate of the average acid consumption per animal per day could be arrived at.

Examination of the tissues.

(*a*) *Bones.* In the rabbits, a femur, fibula and tibia, humerus, radius and ulna were dissected out, freed as far as possible from connective tissue and analysed separately for ash, fat, water, organic residue, calcium and phosphorus. No specific difference was, however, found in the composition of the different bones, and the results for the femur, fibula and tibia are combined and presented together as typical of the bone composition in each animal.

In the rats all the leg bones of the four legs in each animal were used for the analyses.

(*b*) *Soft tissues.* The muscles of the hind legs of the rabbits were removed and freed as far as possible from fascia (no attempt was made to free the muscles from blood vessels, nerves, etc.). The water content was determined by drying at 110° for 48 hours. The muscles were then ashed and the total potassium and sodium were estimated by the method of Husband and Godden [1927]. By this method the total potassium and sodium were weighed as the

mixed sulphates. In the mixture the sulphate was determined and from this the base equivalent to potassium + sodium was calculated as cc. of normal base per 100 g. of muscle tissue. The heart, lungs, kidneys and liver were weighed together, and the water and total potassium + sodium were determined on these tissues as for the muscles.

In the case of the rats it was not practicable to make these determinations on the muscles alone, and the analyses were made on the leg muscles and organs taken together.

Experiments on rabbits.

Three experiments were carried out on rabbits from four litters. Only one rabbit was available from litter 3 which was about the same age as litter 2, but of a larger breed. The results are shown in Table I.

The animals receiving acid either lost weight whilst their controls remained steady, or gained weight much less rapidly than their controls. There were two exceptions. In litter 2, the control animal lost 50 g. while the experimental animal lost 150 g. and in litter 4 one animal receiving acid grew as well as its controls. The control of litter 2 was not suffering from any specific disease and its failure to grow was unaccounted for. The ratio of final bone weight to body-weight indicated that the growth of bone had been rather less retarded than body growth, *e.g.* in litter 4 the final bone weight expressed as a percentage of final body-weight was 0·64 for the two controls, and 0·65 for the experimental animal which grew well, and 0·75 and 0·71 for the experimental animals which showed diminished growth.

The only significant changes in the chemical composition of the bones of the acid group were the increase in the percentage of water and decrease in the percentage of organic matter. Where analyses of fat were made it was found that the decrease in organic matter occurred in the fatty moiety. The control of litter 2 had a bone composition which, like its body growth, was typical rather of the acid than the control group, while the member of litter 4, which grew as well as its controls, had bones of composition similar to those of the controls.

It would therefore seem that the most striking effect of acid ingestion in rabbits is a loss of weight or diminution in growth rate, and that the influence on bone composition is merely that of marked malnutrition (*i.e.* loss of fat and increase in water of bone [Korenchevsky, 1922; Jackson, 1925]). The two animals in the fourth litter, in which growth was retarded, showed a very slight reduction in ash and calcium content of the bone as compared with their controls, but the significance of this is doubtful.

Analyses of the tissues. Table I gives the water content, and the base content of the fresh and dry muscles and organs. From these figures it would appear that there was a slight but definite reduction in base content (sodium + potassium) in fresh muscle in rabbits after the ingestion of acid. This difference was not regularly found in the dry tissues, owing to variations in the water content.

Table I.

Litter	Duration of exp. days	Amount of *N* HCl administered cc.	Weight (kg.) Initial	Weight (kg.) Final	Bone wt. (g.)	Bone analysis: Ash % (A)	Water %	Fat %	Organic residue % (R)	Ca %	P %	Ca/P	Soft tissues: Base equiv. (cc. *N* alkali) per 100 g. muscle: Fresh	Dry	100 g. organs: Fresh	Dry	Water %: Muscle	Organs
1	17	0	0·8	0·8	6·714	26·7	39·2	13·8	20·2	9·6	—	—	13·0	51·4	12·5	46·0	74·8	72·8
	17	45	0·9	0·8	7·151	25·7	50·1	5·0	19·2	9·3	—	—	11·0	43·8	10·4	43·4	74·8	76·1
2	28	0	0·5	0·45	4·400	21·0	58·1	1·4	19·4	7·7	4·2	1·85	12·9	61·1	12·4	51·2	78·9	75·8
	28	120–150	0·6	0·45	5·108	20·6	59·9	0·7	18·8	7·5	4·1	1·85	11·3	48·8	13·5	60·3	77·0	77·6
3	28	120–150	0·9	0·9	6·668	28·6	48·4	—	23·0*	10·5	5·2	2·00	14·2	62·0	11·1	42·9	77·1	74·1
4	32–35	0	0·25	0·65	4·131	19·0	47·8	—	33·2*	6·5	3·6	1·82	14·7	54·4	14·0	50·0	73·0	72·0
	32–35	0	0·20	0·55	3·515	19·0	52·3	—	28·7*	6·5	3·6	1·82	14·5	64·2	12·5	53·2	77·4	76·5
	32–35	150–200	0·20	0·40	2·994	16·8	67·9	—	15·3*	5·9	3·3	1·80	12·9	64·4	13·1	59·5	80·0	78·0
	32–35	150–200	0·25	0·50	3·546	15·7	66·8	—	17·5*	5·4	3·0	1·82	11·7	54·4	11·0	54·4	78·5	79·8
	32–35	150–200	0·30	0·60	3·881	22·0	50·4	—	27·6*	7·7	4·0	1·92	8·6	39·6	9·8	35·5	78·3	72·4

* Organic residue + fat.

Table II.

Litter	Duration of exp. days	Amount of *N* HCl administered cc.	Final body-weight (g.)	Bone weight (g.)	Bone analysis: Ash % (A)	Water %	Fat %	Organic residue % (R)	Ca %	P %	Ca/P	A/R	Soft tissues: Base equiv. (cc. *N* alkali) per 100 g. fresh tissues	Water %
A	70	0	118	2·461	38·9	31·3	6·7	23·1	14·5	7·2	2·03	1·69	12·5	73·9
	70	0	114	2·510	34·5	37·5	5·6	22·4	13·0	6·3	2·05	1·54	12·1	75·3
	70	0	110	2·269	39·8	31·8	5·2	23·2	15·4	7·3	2·10	1·72	13·0	72·6
	70	0	104	2·313	37·8	33·4	6·5	21·8	14·3	7·0	2·03	1·73	12·3	73·2
	Mean	...	112	2·388	37·8	33·5	6·0	22·6	14·3	6·95	2·05	1·67	12·5	73·7
A	70	100–200												
	Mean (4 rats) ...		74	1·971	36·7	34·8	4·0	24·5	14·0	6·90	2·02	1·50	13·8	74·0
B	60	0												
	Mean (4 rats) ...		105	1·944	37·6	34·4	4·9	23·0	14·7	7·0	2·10	1·64	12·4	73·6
B	60	100–200												
	Mean (4 rats) ...		83	1·745	36·9	34·8	4·1	24·2	14·4	7·0	2·05	1·52	12·9	74·1

The variations in the figures for the rats in the control group, litter A, indicate the order of the variations found in the different groups.

There was not, however, evidence of regular reduction in water content of muscle after prolonged ingestion of acid, as might be expected from the diuresis following the ingestion of small quantities of acid.

In the organs there was no regular decrease in base content either in fresh or dry tissues. Again, the water content of the organs from the acid group was usually though not invariably higher than in the controls. (The liver and kidneys of the experimental animals always appeared gorged with blood after the death of the animals.) The analyses of the tissues of the one animal which grew as well as the controls (litter 4) indicated a much more considerable withdrawal of base from the soft tissues than in any of the others, although the bones of this animal were less affected by the acid ingestion.

From these analyses it would therefore appear that acid ingestion in rabbits, as found by Goto [1918], was followed by a withdrawal of base from the muscles. There was no evidence that a withdrawal of base from the organs usually occurred, although from the one rabbit which grew well it may be deduced that such a withdrawal may occur in certain animals under certain conditions. The feeding of the rabbits may have somewhat complicated their response to the acid feeding. As cabbage was supplied *ad libitum* it is possible that some animals may have chosen to eat more than others. This may have accounted for the variations found in the acid group of the fourth litter.

Experiments on rats.

Two litters, each containing eight rats, were used. Four rats from each litter were given acid and four were kept as controls. Both litters contained even numbers of the sexes. The rats each received about 12 cc. of acid daily (0·1 *N* HCl) at the beginning of the experiment. This was gradually increased until at the end of 7 weeks they were each receiving 50 cc. of 0·1 *N* acid daily, and then for the last 2 weeks they were given 50 cc. of 0·2 *N* acid each per day. After the dose of acid reached 25 cc. 0·1 *N* per day, the appetites of the animals fell off and the food supply to the controls was gradually limited to approximate to the amount actually consumed by the experimental groups. This brought the rate of growth of the control animals well below the average for their age, but made it possible to determine the influence of the simple ingestion of acid by the experimental rats, uncomplicated by differences in food intake. After 9 weeks on experiment the animals died, the controls were killed and the bones and tissues were examined as previously described.

The effect of acid ingestion on bone and body growth in rats. Table II shows the final body-weight, bone weight and bone composition of the rats receiving acid and of their controls. The bones (of the four legs) were on the whole slightly smaller in the groups receiving acid than in the control groups. The final body-weights were markedly smaller in the acid than in the control groups with the exception of one rat in litter B. Some of the rats in the experimental group grew as fast as the controls at first, and then lost weight rapidly in the final 2 weeks of the experiment; others grew more slowly than

the controls throughout the whole period. The average bone weights of the groups expressed as percentages of the maximum body-weights were: litter A, acid group 2·08, controls 2·13; litter B, acid group 1·96, controls 1·85. This would seem to indicate that bone growth had been approximately proportional to body growth in both groups and that the final loss of weight in some of the experimental animals had not been accompanied by appreciable breakdown of bone. On the whole, however, both bone and body growth had been slightly retarded in the acid groups.

Bone composition. From Table II it may also be noted that the ingestion of considerable quantities of acid was without appreciable effect on the chemical composition of the bones. The percentages of calcium and phosphorus in the ash of the bone did not indicate any differences between the bones of the two groups. The A/R ratio which may be taken as a measure of the degree of ossification of the bone [Chick and Roscoe, 1926] was slightly but definitely decreased in the acid group, although the animal in litter B, which grew as well as its controls, also had an A/R ratio which fell into the range of the control group. Each of the control groups also contained one animal of which the A/R ratio was typical rather of the acid than control groups. Despite this variation the difference between the two groups, although slight, would seem to indicate a definite retardation in the rate of ossification of bone in the acid group, particularly during the last few weeks of the experiment. The marked reduction in fat content of the bone which was so typical of the rabbits receiving acid was not found in the case of the rats on the acid diet.

Analysis of the soft tissues. The soft tissues of the animals in the acid group showed no loss of water as compared with their controls. There was no diminution in the percentage of base in the wet tissues of the acid group (Table II). On the contrary, there was a slight increase in this respect in all the animals receiving acid, with the one exception of the animal in litter B which grew normally. Not only in growth but in composition of bone and tissues this animal appeared to be similar to the control rather than the acid groups.

Discussion.

While in the muscles of rabbits receiving acid there seemed to be a slight reduction in base content (potassium + sodium), this was not the case for the organs of the rabbit, nor would it appear to be the case for the muscles and organs of the rat. The most striking influence of the ingestion of acid was the chronic malnutrition of the animals resulting in retarded growth and in six out of seven rats considerable loss of weight. The effect on the bones of rabbits was also that typical of severe malnutrition. In the case of the rats, it may be concluded from the A/R ratio of the different groups that the ossification of bone was retarded when the ingestion of acid was so great that actual loss of weight occurred, but there was no sign in any animal of a differential withdrawal of calcium from bone, resulting in softened bones, nor of a breakdown

of osseous tissue resulting in bones which were of small weight compared with the weight of the animal. The tissues of the animals in the acid group showed no signs of dehydration, as might have been expected from the diuresis shown in the short period experiments of Fiske and his co-workers [1926] and Gamble and Ross [1923].

If it were assumed that the excess acid was carried in the blood as ammonium chloride, it might be concluded that the retardation of growth and final loss of weight was due to the toxicity of this ammonium chloride. During the last weeks of the experiment the animals daily ingested enough acid to produce a lethal dose of ammonium chloride [Underhill and Kapsinow, 1922], but the effect of the latter would be minimised by being slowly produced and by being excreted as formed. Gamble and Ross [1923], however, showed that the ingestion of ammonium chloride had the same influence on the composition of blood and urine (p_H, serum-bicarbonate, ammonia of urine, etc.) as the ingestion of an equivalent quantity of hydrochloric acid. They concluded that the ammonium chloride was dissociated and that the ammonia was carried in the blood as some neutral substance, while the hydrochloric acid was carried in combination with the fixed base of the serum, and the serum-bicarbonate was lowered. In the case of prolonged ingestion of acid, however, this mechanism might prove inadequate, as plasma-bicarbonate could not be lowered beyond a certain point if tissue CO_2 was to be removed satisfactorily. An increase in plasma-base would therefore be necessary to permit of the transport of the excess acid. (This increase in total plasma-base might be achieved by increasing the plasma volume without altering the concentration of base in the plasma.) The net result would be a greater call on the bases absorbed from the food to supply plasma-base, and a limitation of base available for tissue building.

From the colloidal nature of cell protoplasm, it would seem probable that the salts present in the tissues play an important part in preserving the electrical equilibria which are the conditions necessary for the normal functioning of the cells, and that, without an adequate supply of salts available for tissue-building, normal growth cannot take place. Hence the limitation of growth where the conditions tend to increased base excretion. In the rats, when the dose of acid was greatly increased towards the end of the experiment, actual breakdown of tissue occurred, presumably to supply the base necessary for the neutralisation and excretion of acid. This liberated, however, more base than was actually necessary for reaction with the acid, and some was retained in the tissues. This would account for the slightly increased base content in the soft tissues of those rats of the acid group in which considerable loss of weight had occurred. This retention of base in the tissues during the rapid breakdown of other tissues may perhaps be comparable to the retention of sodium in starvation [Lusk, 1928].

SUMMARY.

(1) Five rabbits on a fixed diet received acid daily for periods varying from 17 to 35 days. Compared with litter-mates on the same diet without acid, these animals showed a reduction in the base content of the muscles, but not of the viscera.

(2) There was no reduction in the percentage ash of the bones, but a marked reduction in the fat content.

(3) All the animals receiving acid showed either retarded growth or actual loss of weight, and suffered from severe malnutrition.

(4) Eight rats on a standard diet received daily doses of acid over a period of 9 weeks.

(5) There was no appreciable reduction in the fat content of the bones, and the ash content was unchanged.

(6) Growth was retarded, and considerable loss of weight occurred when the dose of acid was increased.

(7) The base content of the soft tissues was increased slightly where death was preceded by marked loss in weight.

The administration of acid and food in all the experiments was carried out by Prof. Burns who suggested this study. The expenses were defrayed by a grant to him from the Medical Research Council to whom I desire to express my thanks.

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Chandler-'26
Bib No. 67

Ind. Jour. Med. Res. 14(1):219-227. 1926.

EFFECTS OF THE ADMINISTRATION OF SUGAR, MAGNESIUM SULPHATE, SODIUM CITRATE AND DILUTE ACID ON THE LIVER DAMAGE DONE BY CARBON TETRACHLORIDE.

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[Received for publication, March 24, 1926.]

DAVIS (1924) carried out a series of experiments on dogs which showed that variations in diet, effective for four days, had a very marked effect on the damage done by carbon tetrachloride to the liver and kidneys. In most of his experiments he used a dosage of 0.4 to 0.5 c.c. of carbon tetrachloride per kilo of body weight. His results showed that starvation for three or four days, or a fat diet for four days, had the effect of greatly increasing the amount of liver damage as compared with that in dogs on a high protein and more particularly a high carbohydrate diet for a similar period of time. He calls attention, however, to the fact that, no matter how small the dosage, or how favourable the dietary conditions, there was always produced some liver injury as indicated by fatty infiltration. He states that glycogen content *per se* may or may not exert any protective influence for the liver, but a liver well stored with glycogen is certainly refractory towards large doses of either chloroform or carbon tetrachloride, and conversely a liver free from glycogen, especially if it contains a plentiful supply of fat, is decidedly susceptible to such protoplasmic poisons.

Since it is very rarely practical, under the conditions under which hookworm treatment, and particularly mass treatments, are given in the field to regulate the diet for even one day, to say nothing of four days, the practical application of Davis' work is very limited, except in so far as the danger of starvation or underfeeding are concerned. It is traditional to starve patients before the administration of anthelmintics, yet in the case of carbon tetrachloride starvation not only is not necessary but is distinctly contra-indicated. We are of the opinion that many of the deaths from carbon tetrachloride in Assam coolies, which brought the drug into such ill repute among tea planters

soon after its introduction, were due to insistence on the preliminary starvation period, the effect of which was increased by cirrhosis of the liver due to chronic alcoholism, and perhaps in some cases habitual undernourishment.

Stimulated by Davis' findings, we have carried out an extensive series of experiments to determine whether or not it would be possible to control the liver and kidney damage, to some extent, by the administration of sugar preliminary to or with the carbon tetrachloride, and also to determine whether the acidity or alkalinity of the intestinal tract had any influence by altering the amount of absorption.

Experiments on Cats.

Since it was impossible to keep dogs in the compound of the school, which is also occupied by a hospital, we hoped to be able to carry out these experiments on cats. According to Hall and Shillinger (1923) cats can be given relatively large doses of carbon tetrachloride, up to 9 c.c. per kilo, without fatal effect. Relying on Hall and Shillinger's findings we began our work by giving a series of six full-grown cats a dose of 0.5 c.c. of carbon tetrachloride, thinking that this dose would be sufficient to produce recognisable symptoms in unprotected cats and yet not be too severe. The carbon tetrachloride we used was the same as that used in our hospital work and is free from carbon bisulphide and phosgene, and conforms to other standards of purity ordinarily laid down for carbon tetrachloride. The drug was given three hours after a normal diet of rice and cooked meat. To our surprise three of these cats died within 48 hours, and two more on the third day. The remaining one, which was ill, was killed on the third day. During the day following treatment, all of the cats lay about, very sluggish and obviously very ill; the respirations were rapid and weak. Towards evening their bodies were cold and the cats were no longer able to stand on their feet. Post-mortem examinations showed the liver in all cases to be severely injured, showing the appearance described by Gardner et al. (1925) as 'trabecular tracery,' the centre of the lobules being sunken and hyperæmic, deep red in colour, surrounded by yellowish fatty zones. This is the same appearance as described by Davis for livers in which central necrosis was observed. The intestinal walls were red and congested, and the upper part of the intestine, and in some instances the stomach, was in most cases well filled with a rusty red slimy substance, apparently altered blood and tissue debris, presumably carried there from the injured liver. Urine was obtained from two of the cats and in each case it contained large quantities of albumen and some bile pigments.

Microscopic examination of the liver showed that practically the entire liver was in a necrotic state, no more than at most a very few cells around the periphery of the lobules being in a viable state. Throughout the necrotic areas there was very extensive fatty infiltration and vacuolation. Examination of the kidney showed a very striking degree of fatty degeneration of the convoluted and zigzag tubules, with desquamation of Henle's loops in some instances, and a sero-sanguinous exudate in Bowman's capsules; the glomeruli had a swollen and vacuolated appearance. In the cat that was killed on the third day the

liver showed slightly less necrosis than in the others and the kidney, although showing a considerable amount of fatty degeneration and cloudy swelling, was obviously in healthier condition than in the case of the other cats. The intestinal wall showed desquamation of the villous epithelium. In two of the cats the suprarenals showed fatty infiltration, giving the cells a foamy appearance, but in the others this organ was apparently uninjured. No changes in the spleen or pancreas were observed.

The result of this trial experiment being so much at variance with results obtained by Hall and Shillinger, and the only difference we could find between their experiments and ours being the age and size of the cats, we repeated the experiment using kittens weighing from 673 to 943 grams, similar to those used by Hall and Shillinger. The results in this case were no better; two of the kittens died on the second day, two on the third day, and one on the fourth. The sixth one, which appeared only slightly ill, was killed on the third day. The macroscopic appearance of the organs was similar to that of the cats in the first series, but there was evident jaundice of the abdominal walls. Microscopically the tissues of the kittens which died on the second and third days resembled those of the first group. The kitten which died on the fourth day showed intense fatty degeneration of the liver and necrosis of about 90 per cent of the liver tissue; the kidneys were in a state of extensive desquamation and necrosis of all the tubules, indicating that the damage was progressive. The kitten which was killed on the third day showed a very moderate degree of liver necrosis, amounting to not more than 40 per cent of the liver tissue, whereas the kidneys showed moderately severe fatty degeneration. It is probable that this kitten would have survived at least for some days.

Experiments 3, 4 and 5 were designed to show whether the administration of sugar on the day of treatment would have any effect in reducing the damage to the liver and kidneys, by filling the liver with glycogen. Cane-sugar was used instead of glucose since, in case sugar administration should prove to be of value and were used in a practical way in the treatment of human beings, cane-sugar would be much cheaper and more practical than glucose. Preliminary experiments showed that 3.4 grams of sugar per kilo, in a 12½ per cent solution, was sufficient to increase the blood-sugar without causing appreciable glycosuria, indicating a maximum absorption of the sugar by the liver; therefore this amount of sugar was used. In experiment 3 the sugar solution was given to six cats by stomach tube five hours before the carbon tetrachloride and again simultaneously with the drug. Bread and milk was allowed half an hour after the first dose of sugar, being removed four hours before the carbon tetrachloride was given. One of these cats escaped, so there were only five left for observation. The results of this experiment were distinctly encouraging. One cat was killed in a dying condition on the second day, one was killed in a dying condition on the fourth day and another one died on that day, another killed in a dying condition on the fifth day, while one was killed in apparently good health on the second day. In other words, death, which had ensued on the second and third days in nine out of twelve of the unprotected cats, was delayed in this series, with one exception, to the fourth and fifth days. The

dying cat killed on the second day showed slightly less liver damage and slightly more severe kidney damage than the unprotected cats. The healthy cat killed on the second day showed about 50 per cent liver necrosis and moderate kidney damage. The cat which died on the fourth day showed liver and kidney damage

TABLE I.

Effect on time of death of cats, due to CCl_4 administration, of sugar, mag. sulph. purge, sodium citrate, hydrochloric acid and size of dose.

Experiment No.	Number of cats.	Sugar several hours before CCl_4.	CCl_4, c.c. per kilo.	Sugar simultaneously with CCl_4.	Mag. sulph. purge.	Sodium citrate 1oz. per kilo of 1% solution.	Hydrochloric acid 27 c.c. of 1% solution.	Died, first day.	Died, second day.	Died, third day.	Died, fourth day.	Died, fifth day.	Survived more than 5 days.	REMARKS.
1	6	—	0·5	—	—	—	—	0	3	2	3	0	0	(One killed 5th day.)
2	6	—	0·5	—	—	—	—	0	2	2	1	0	0	(One killed 3rd day.)
3	6	+	0·5	+	—	—	—	0	1	0	2	1	0	(One escaped, 1 killed 2nd day, healthy.)
4	6	+	0·5	—	—	—	—	1	5	0	0	..	0	
and 6	12	—	0·5	+	—	—	—	1	3	5	1	..	1	(One killed 4th day, healthy.)
7	8	+	0·5	—	+	—	—	1	2	2	0	..	3	
8	6	+	0·5	+	+	—	—	0	2	1	1	..	2	
9	6	—	0·25	—	+	—	—	0	2	2	0	..	1	(One died during feeding.)
10	6	+	0·25	—	+	—	—	1	2	1	2	0	0	
11	8	—	0·25	—	—	+	—	..	1	..	..	..	..	(Seven vomited, no record.)
12	10	—	0·25	+	—	+	—	3	1	1	0	0	5	(Of the survivors, 3 died on 6th, 11th and 14th days.)
13	6	—	0·25	—	—	—	+	0	4	2	0	0	0	
14	11	—	4·00	—	—	—	—	3	4	4	0	0	1	(Nine vomited; survivor died 8th day.)
15	4	—	4·00	(in milk)		—	—	0	2	2	0	0	0	

similar to that of the cat which was dying on the second day. The cat which was killed in a dying condition on the fifth day showed relatively little necrosis of the liver, amounting to not more than 25 per cent and very little fatty degeneration, whereas the kidney was in a state of severe fatty degeneration with desquamation of many of the tubules. The experiment seemed to indicate

clearly that the sugar had a protective effect on the liver but none on the kidney, and that the delayed death was due to renal rather than to hepatic injury. Experiment 4 was similar to 3 except that only the preliminary dose of sugar, five hours before the carbon tetrachloride, was administered. The results of this experiment were disappointing. One of the cats died within 24 hours and all the other five on the second day. These cats all showed from 90 per cent to 100 per cent central necrosis of the liver as well as fatty vacuolation, together with relatively severe fatty degeneration of the kidneys which, however, varied somewhat in different individuals. In experiment 5 six cats were given a single dose of sugar concomitantly with the carbon tetrachloride, and slightly better results were obtained. One cat died on the day following treatment, one on the second, two on the third, one on the fourth and one not until the ninth. In all of these the kidney damage was unusually severe, whereas the liver damage was only moderately severe. The fact that many of these cats refused food after treatment led us to believe that better results might be obtained if another dose of sugar were given on the day following treatment. Of six cats so treated (experiment 6), two died on the second day, three on the third, and one was killed in apparently healthy condition on the fourth. Microscopic examination of the liver and kidneys of the cats which died showed extensive damage to both organs, fully as great as in experiment 5. The cat killed on the fourth day showed about 50 per cent liver necrosis, evidently in process of repair, and moderate fatty degeneration of the kidneys.

In experiment 7 we tried the effect of one dram of saturated solution of magnesium sulphate given to four cats two hours after carbon tetrachloride, and to four others concomitantly with the drug. No difference in the results of the two series are evident. One of the eight died on the first day, two on the second, two on the third, one on the fourth, and two survived. Taking the group as a whole, the saline purge seems to have had a beneficial effect, although the liver and kidney damage in the cats which died was quite as severe as without the purge. No differential protection between liver and kidney was observed, comparable with that in experiment 3. No better results were obtained when this same experiment was repeated (experiment 9) using only 0.25 c.c. of carbon tetrachloride per kilo. In this experiment one cat died during stomach feeding, some of the drug presumably having gotten into the trachea. Of the other five two died on the second day, two on the third and one survived. The liver and kidney damage in the cats which died was quite as severe as in those which received 0.5 c.c. per kilo. In experiment No. 8 six cats were given sugar before and simultaneously with carbon tetrachloride (0.5 c.c. per kilo) and a saline purge two hours afterwards. In this experiment two cats died on the second day, one on the third and one on the fourth and two survived. These results are neither better nor worse than when a single dose of sugar was given.

In experiment 10, we fed six cats with a preliminary dose of sugar, 0.25 c.c. per kilo of carbon tetrachloride and a saline purge. Of this series one cat died on the first day, two on the second, one on the third, and two on

Experiment 11 was planned to ascertain whether an alkaline condition of the intestine would have any effect in increasing or decreasing the toxicity of carbon tetrachloride by modifying the absorption. The cats were given one ounce per kilo of a 1 per cent solution of sodium citrate and 0.25 c.c. of carbon tetrachloride per kilo. The results of this experiment were inconclusive since five of the cats vomited. The one which did not vomit died on the second day with severely damaged liver and kidneys. Experiment No. 12 was a repetition except that a full dose of sugar was added to the solution. Of ten cats six vomited. Of those which did not vomit, one died in 24 hours, one in six days and two survived. Of those which vomited, one escaped, two died after 24 hours, one on the second day and one on the third and one survived. We think it probable that the inhalation of carbon tetrachloride from vomitus in the mouth to some extent counterbalances the loss of carbon tetrachloride from the stomach. Death seldom occurs in the first 24 hours except in cats which vomit. It seems to us that this experiment indicates a favourable effect of alkalinity of the intestine during administration of carbon tetrachloride.

Experiments 13 was designed to show the effect of increased acidity. Six cats were given 27 c.c. of a 1 per cent solution of hydrochloric acid together with a full dose of sugar, but all of them vomited. Four of these cats died on the second day and two on the third.

Experiments 14 and 15 were planned to ascertain whether when large doses of carbon tetrachloride were given, the anæsthetizing effect on the digestive system would be sufficient to prevent enough absorption to cause death. Eleven cats were fed with 4 c.c. per kilo, but nine vomited. Of the eleven, three died on the first day, four on the second day, two on the third day, one on the fifth, and one survived until the eighth day. The two which did not vomit, died on the first and third days.

In experiment 15 four other cats were given 4 c.c. emulsified in milk; two vomited. Two of these cats died on the second and two on the third days. Apparently, therefore, a very large dose is not more rapidly fatal than a small dose, but it is impossible to say how much of the carbon tetrachloride was eliminated by the cats which vomited.

Experiments on Dogs.

Since the extreme susceptibility of cats to carbon tetrachloride made it difficult to judge the effect of our attempts at protection, we determined to repeat some of our experiments on dogs, though we had to rely on a menial to look after them and feed them, outside the school compound.

We performed six experiments using from three to six dogs in each one. We adopted 2 c.c. of carbon tetrachloride per kilo as our standard experimental dose. The dogs were fed on a mixture of rice and cooked meat, the menial who cared for them being warned not to feed them with fat, but we cannot be sure that our warning was heeded.

We were surprised, in view of the striking tolerance shown by dogs for carbon tetrachloride, to find what a high degree of liver damage was done by

the doses administered, and were disappointed to find that administration of sugar a few hours before the carbon tetrachloride, or simultaneously with it, or both, with or without a purge of magnesium sulphate, had no apparent effect in modifying the amount of damage, although the individual susceptibility was extremely variable. We were also surprised to find that dogs with as high as 75 per cent of the liver tissue necrosed showed no obvious symptoms of illness. Only when the necrosis reached 90 per cent or more did the animals become ill and die. The results of the experiments are shown in Table II. The average amount of liver necrosis in the dogs receiving two doses of sugar and in those receiving one simultaneous dose of sugar and also a saline purge, is slightly less than in the other groups, but we doubt if any significance can be attached to this.

TABLE II.

Effect on amount of liver necrosis in dogs, due to CCl_4 administration, of sugar, hydrochloric acid, and mag. sulph. purge.

Experiment No.	Number of dogs.	CCl_4 per kilo.	Sugar several hours before CCl_4.	Sugar simultaneously with CCl_4.	Hydrochloric acid 27c.c. of 1% solution.	Mag. sulph. purge.	Per cent of liver necrosis in each animal.
1	3	2 c.c.	−	−	−	−	60 75, 70.
2.	3	2 c.c	−	−	+	−	75, 100, 75.
3	3	2 c.c.	+	−	−	−	75, 80, 40.
4	6	2 c.c.	+	+	−	−	30, 30, 50, 60, 65 (one escaped).
5	6	2 c.c.	−	+	−	−	95, 90, 75, 55, 60, 65.
6	3	2 c.c.	−	+	−	+	65, 60, 55.

In all cases, even in dogs with 90 per cent to 100 per cent liver necrosis, the kidney damage was negligible as compared with that in the cats, but a slight degree of fatty infiltration and cloudy swelling was invariably present.

CONCLUSIONS.

We have established the fact that carbon tetrachloride is extremely toxic to cats, very much more so than to dogs. The liver of cats is damaged in the same way as in dogs, but more severely, and in addition the kidney undergoes a profound degree of degeneration accompanied by copious albumen in the urine. Practically the same results were obtained from 0.25 c.c., 0.5 and 4 c.c. per kilo.

Our experiments indicate that administration of sugar simultaneously with carbon tetrachloride tends to delay death for a day or two; sugar given a few

hours before carbon tetrachloride without a second dose simultaneously with it does not appear to have any protective effect. Administration of sodium citrate, tending to increase the alkalinity of the intestine, somewhat reduces the toxicity, while administration of dilute hydrochloride acid increases it. A magnesium sulphate purge also tends to reduce toxicity.

In dogs we found a very considerable individual difference in susceptibility to carbon tetrachloride, but always much less than in cats. A dose of 2 c.c. per kilo caused a central necrosis of the liver lobules usually amounting to 50 per cent to 75 per cent liver destruction without obvious symptoms. We could demonstrate no significant protection resulting from administration of sugar before or with carbon tetrachloride.

In view of these results we doubt whether administration of sugar, for example in the form of syrup, toffee or other sweet confections, along with carbon tetrachloride would have any advantage in controlling the safety of the administration of this drug, and we think that avoidance of starvation or a fat diet during its administration and encouragement of a carbohydrate and protein diet, is sufficient. It is probable that administration of sodium citrate or sodium bicarbonate along with carbon tetrachloride would tend to reduce the amount of absorption, and thereby lessen the danger in its use. A purge undoubtedly helps in lowering the amount of absorption by hastening the elimination of the drug.

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Code-'63
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J. Physiol. 166:110-119. 1963.

J. Physiol. (1963), 166, *pp.* 110–
With 11 *text-figures*
Printed in Great Britain

THE INFLUENCE OF ACID ON THE GASTRIC ABSORPTION OF WATER, SODIUM AND POTASSIUM

BY C. F. CODE, J. A. HIGGINS, J. C. MOLL, A. L. ORVIS AND J. F. SCHOLER

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(*Received* 29 *June* 1962)

Cope and his co-workers (Cope, Blatt & Ball, 1943; Cope, Cohn & Brenizer, 1943) demonstrated years ago that in pouches of the gastric corpus and fundus of dogs absorption of radiosodium is slow. They found that the rate was particularly retarded when these pouches were secreting acid. Radiosodium was absorbed much more rapidly from pouches of the antrum of the stomach, and the secretory activity of these pouches had no effect on the rate. Also, labelled water moved more quickly from the contents of the pouches to the blood stream than did sodium; and this movement of water was independent of the secretory activity of the pouches. Reitemeier, Code & Orvis (1957) found that the gastric mucosa of healthy human beings offered a complete barrier to the passage of sodium from the gastric contents into the blood while the water in which the radiosodium was dissolved moved into the blood stream at a rate of 2–3 ml./100 ml./min.

The investigation to be reported was undertaken (1) to determine whether the gastric mucosa of the dog, studied under conditions different from those employed by Cope and his co-workers, presents a complete barrier to the absorption of sodium, as does that of the human being; (2) to explore the relationship of hydrochloric acid to the maintenance of this barrier; and (3) to test whether the absorption of water or potassium from the stomach is influenced by the secretion or addition of acid to the contents.

Abstracts of this study have been published by Code, Scholer, Orvis & Higgins (1955), Code, Higgins, Moll, Scholer & Orvis (1956), Code, Higgins & Orvis (1956), Moll, Code & Orvis (1956).

METHODS

Healthy mongrel dogs were used. They were kept without food for 24–48 hr and then anaesthetized with thiopental sodium. With aseptic surgical technique, the abdomen was entered through the mid line, the gastro-oesophageal junction was tied with tape, and the duodenum was entered in its first part through a longitudinal incision on the antimesenteric border. A perforated plastic tube with a spool fixed just proximal to the perforations was passed through this incision into the stomach (Fig. 1). A strong ligature was tied over the

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spool at the junction of the corpus and the antrum of the stomach. The fundus and corpus of the stomach were thus sealed off from the oesophagus above and the antrum below. The exclusion of antral mucosa from the isolated segment was verified in each dog at necropsy. The unperforated portion of the tube was brought to the surface through the duodenal and abdominal incisions. After these incisions had been closed, a special indwelling needle with a fitted stylet was threaded into the femoral artery through a branch, so that a free flow of blood passed the tip of the needle at all times.

Upon completion of the operative procedures the stomach was rinsed with warm physiological saline solution until the returning fluid was clear. A rest period of at least 30 min was allowed, at the end of which the stomach was emptied and the pH of the contents determined, by means of a glass electrode. Tests of the pH of the contents were repeated each 15–30 min until the pH values indicated that the mucosa was at rest or in a constant or steady secretory state. Then a test of absorption was started.

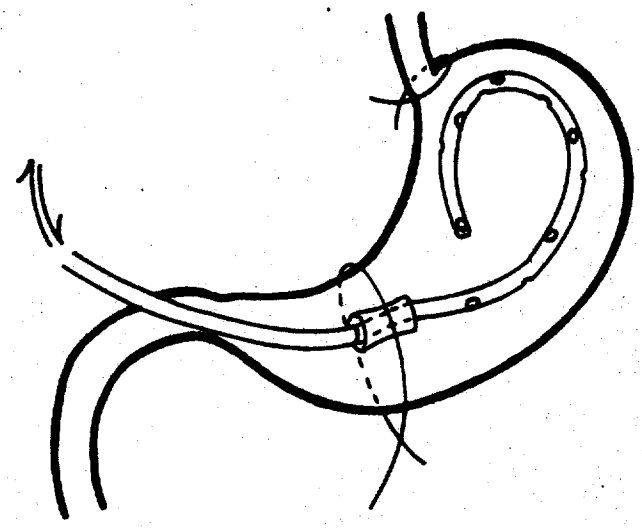

Fig. 1. Isolation of corpus and fundus of stomach as separate compartment by ligature tied at gastro-oesophageal junction and over spool at junction of antrum and corpus. Solutions were introduced and withdrawn from this compartment through a plastic tube passed through spool.

The solution placed in the stomach always contained sodium chloride. Occasionally this was isotonic but usually the solution was hypotonic. D_2O, THO, ^{22}Na, and ^{42}K were mixed with the test solution before it was placed in the stomach or during the course of the test; and acid, alkali, sodium chloride, or potassium chloride was added likewise before or during the tests, depending upon the nature of the experiment. Samples of arterial blood were withdrawn every 2 min during the most active phases of the absorption of the isotopes and at longer intervals as their rates of absorption declined. For the determination of the concentration of the isotopes at equilibrium, samples of blood were taken 2 hr after completion of the absorption of water and sodium and 8 hr after completion of the absorption of potassium. The rates of absorption of the isotopes were calculated according to the method of Scholer & Code (1954). Mean arterial disappearance rates of labelled water, sodium, and potassium reported for dogs in a previous study were used in the calculations (Code, Moll & Orvis, unpublished). When appropriate, mean rates of absorption were calculated for labelled water, sodium, and potassium. The values during each successive period in the progress of a test were expressed as percentages of the isotope remaining in the stomach, and

these were plotted against time on semi-logarithmic paper. The slopes of the mean val gave the mean rates of absorption per minute.

The research progressed in a series of steps with observations of a similar nature be made on at least five dogs at each stage. The results were found to be most clearly depic by graphic presentation of single experiments representative of each stage of the study.

RESULTS

Absorption of sodium and water

When labelled sodium and water were placed in a stomach with neutr contents, the sodium was absorbed slowly while the water in which it w dissolved passed quickly into the blood stream (Fig. 2). The mean rate sodium absorption in 12 experiments in which the contents were neutr was 0·47 g/100 g/min. If, before the addition of the isotopes, gastr

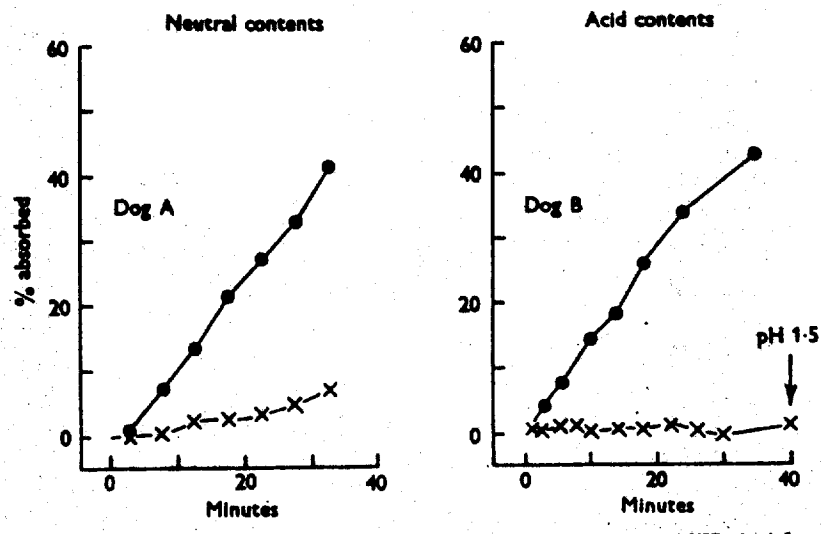

Fig. 2. Contrast of absorption by two different dogs of D_2O (●) and ^{24}Na (×) from neutral and acid gastric contents. Some ^{24}Na was absorbed when contents were neutral, none or practically none when they were acid, while absorption of D_2O was not affected by difference in pH.

secretion of acid was stimulated by repeated subcutaneous injection of histamine (0·3–0·4 mg subcutaneously every 10–15 min), little or no sodium passed from the contents of the stomach to the blood stream, although the water in which it was dissolved was absorbed at its usual rate (Fig. 2). In other tests the absorption of sodium from neutral gastric contents was promptly interrupted when during the course of the experiment the pH of the contents was changed to acid by stimulation of gastric secretion (Fig. 3). If, as often happened, such experiments were so prolonged that all or nearly all the D_2O placed initially in the stomach had disappeared by

the time acidification was accomplished, then tritiated water was added to the contents to allow a second determination of the rate of absorption of water (Fig. 3). Tests of this nature clearly indicated that the secretion of acid resulted in a complete blockade of the movement of sodium from the gastric contents to the blood stream without significantly altering the rate of absorption of water.

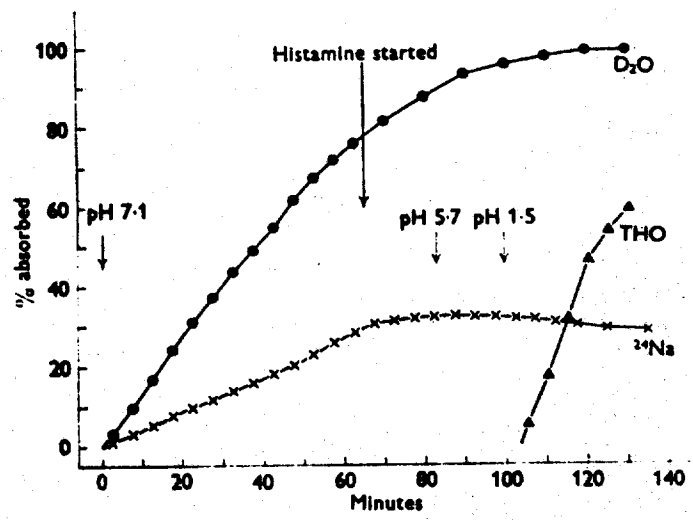

Fig. 3. Interruption of absorption of sodium from gastric contents when secretion of acid was stimulated by injection of histamine. Absorption of water, tested by D_2O when contents were neutral and by THO when contents were acid, was little affected.

The next series of tests was designed to answer the question, does the barrier to the absorption of sodium depend upon the secretion of hydrochloric acid or simply upon the presence of the acid in the contents? The addition of extrinsic hydrochloric acid to the gastric contents blocked the absorption of sodium as effectively as the stimulation of its secretion (Fig. 4). Again, the absorption of water was not affected (Fig. 4). Restoration of neutrality of the contents by the addition of sodium hydroxide caused resumption of absorption of sodium but at a slower rate than when the contents were originally neutral (Fig. 5).

In the third group of experiments the secretion of hydrochloric acid was maintained by repeated subcutaneous injections of histamine and the gastric contents were alkalinized during the course of the test by frequent additions of sodium hydroxide. The absorption of sodium, absent or negligible at the outset while the contents were acid, was induced when the contents were alkalinized; but when addition of sodium hydroxide

was discontinued and the acid reaction of the contents was allowed to return, the absorption of sodium was not stopped (Fig. 6). These results indicated that the relative concentration of sodium and hydrogen ions, rather than the pH, was the determining factor in the absorption of sodium. Experiments were therefore done to test this hypothesis.

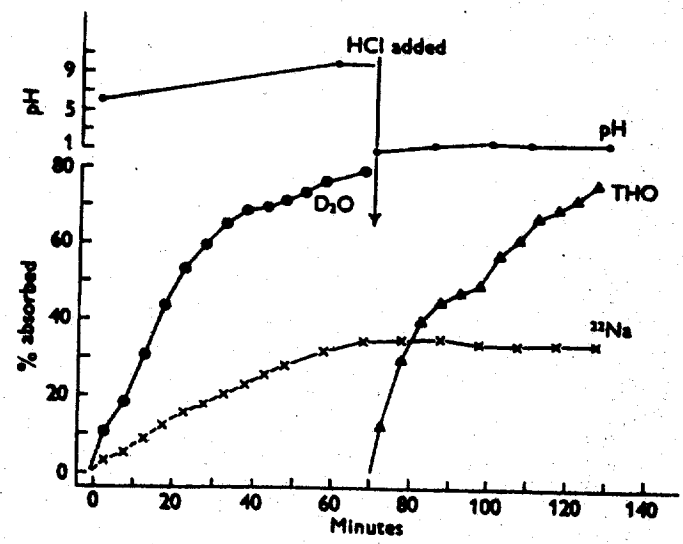

Fig. 4. Interruption of absorption of sodium from stomach by addition of hydrochloric acid to contents.

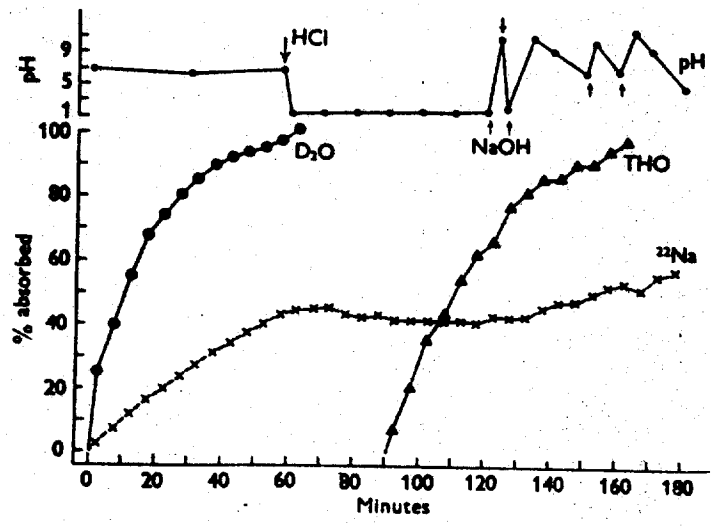

Fig. 5. Alterations in rate of absorption of ^{22}Na from stomach with changes in pH of contents produced by additions of hydrochloric acid and sodium hydroxide.

In the presence of hydrogen ions of extrinsic origin in high concentrations, the addition of large quantities of sodium chloride to the gastric contents always induced the absorption of sodium, even though the pH of the contents remained acid (Fig. 7). Tests with less extreme concentrations of sodium chloride than those illustrated in Fig. 7 verified the results and showed that there was a relationship between the percentage of sodium

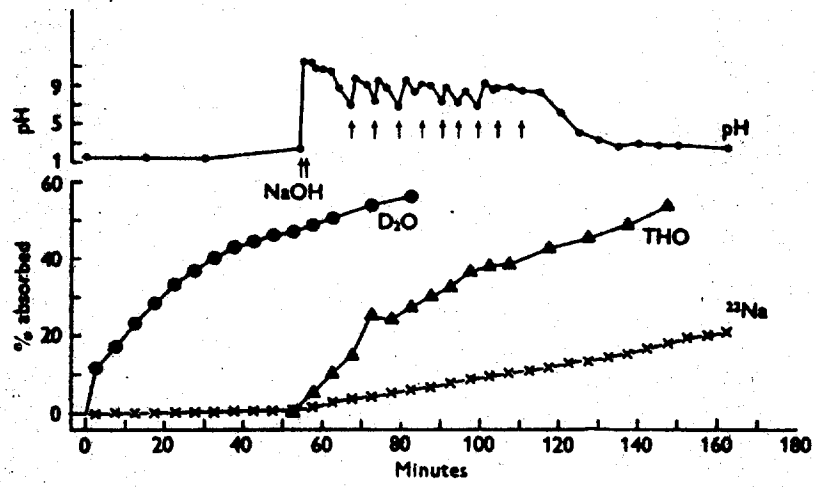

Fig. 6. Changes in rate of absorption of ^{22}Na from stomach during continuous stimulation of secretion of acid by injections of histamine. When contents were alkalinized by addition of sodium hydroxide, absorption of ^{22}Na became appreciable; reacidification by discontinuing additions of sodium hydroxide did not abolish absorption of ^{22}Na.

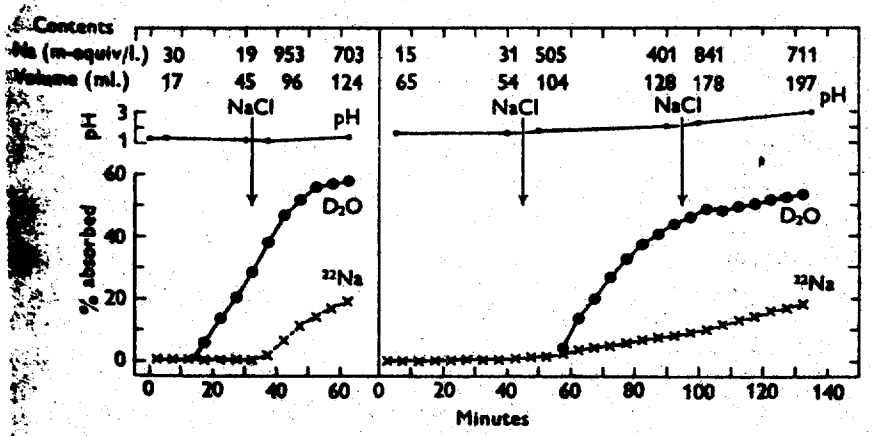

Fig. 7. Absorption of ^{22}Na induced by addition of sodium chloride to acid gastric contents, acid pH of which was maintained (left panel, intrinsic HCl) by continuous HCl secretion by histamine stimulation, or (right panel, extrinsic HCl) by addition of HCl (acidified at outset with 0·1 N-HCl).

absorbed and the ratio of sodium to hydrogen ions in the contents (Fig. However, until the concentration of sodium ions exceeded the concentration of hydrogen ions by a ratio of about 10:1, there was little or movement of sodium from gastric contents to blood.

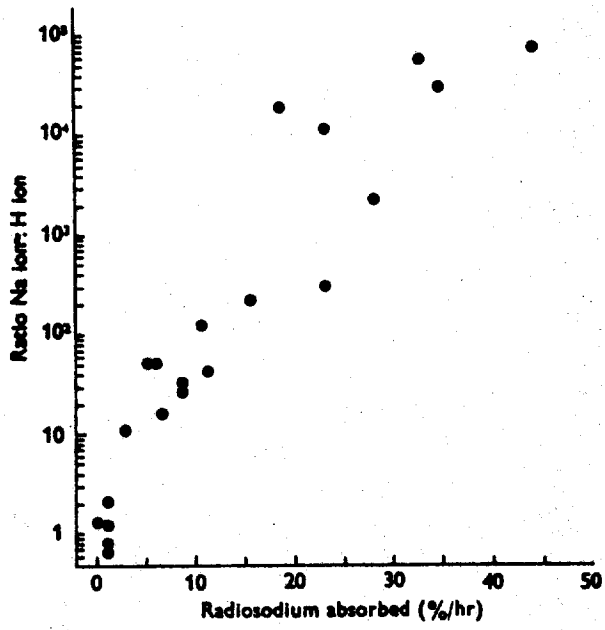

Fig. 8. Relation of rate of absorption of radiosodium to ratio of concentrations of sodium ions to hydrogen ions in contents of stomachs not secreting acid. (Changing concentrations of both ions when mucosa was secreting acid did not allow calculation of accurate ratios. Logarithmic ordinate.)

Through all these tests acidification of the gastric contents by intrinsic or extrinsic HCl produced little if any change in the absorption of water. Certainly acidification did not slow the absorption of water, for the mean rate when the contents were acid ($N = 27$) was slightly faster than when the contents were neutral ($N = 21$). The difference, however, was not significant and therefore the values were combined to yield 1·65 %/min as the mean rate of gastric absorption of water (Fig. 9). The experiments showed too that, within the ranges tested, the absorption of water was independent of the concentration of sodium in the gastric contents. Changing the reaction of the contents from neutral to acid by initiation of secretion or by addition of hydrochloric acid also did not affect the rate of absorption of potassium (Fig. 10). In some experiments marked changes in the concentration of sodium or potassium in the contents seemed to affect the rates of absorption of the other cation, but the number of such

experiments was not sufficient to allow conclusions and there was some indication that these ions in high concentration damaged the mucosa.

In a final series of tests, both labelled sodium and potassium were placed in a stomach with neutral contents. Acidification by initiation of the secretion of acid promptly stopped the absorption of sodium, while the rates of passage of water and potassium from the contents to blood were not affected (Fig. 11).

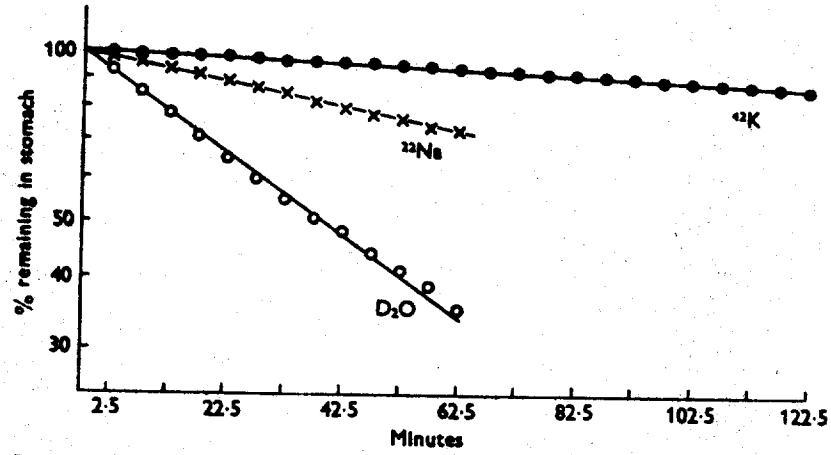

Fig. 9. Mean rates of absorption of D_2O (neutral and acid contents; mean rate 1·65 %/min, $n = 48$); ^{22}Na (neutral contents; mean rate 0·47 %/min, $n = 12$); and ^{42}K (neutral and acid contents, mean rate 0·11% /min, $n = 8$) from stomachs of dogs. Logarithmic ordinate.

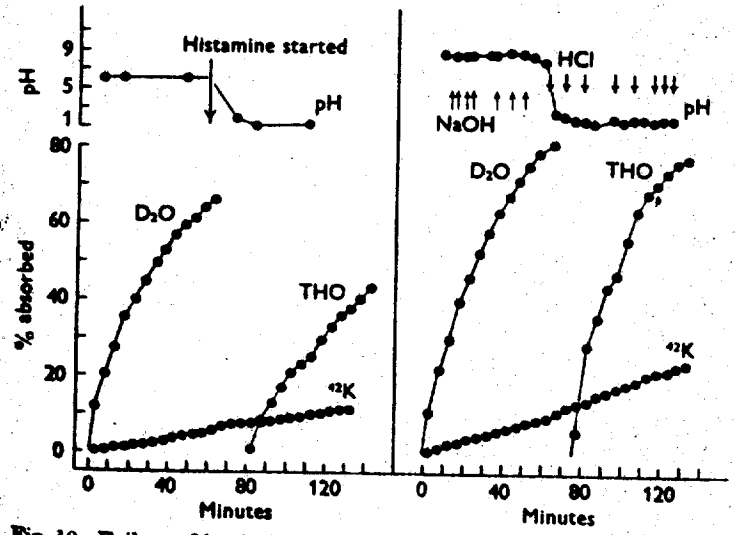

Fig. 10. Failure of intrinsic and extrinsic hydrochloric acid to affect rate of absorption of ^{42}K from the stomachs of dogs.

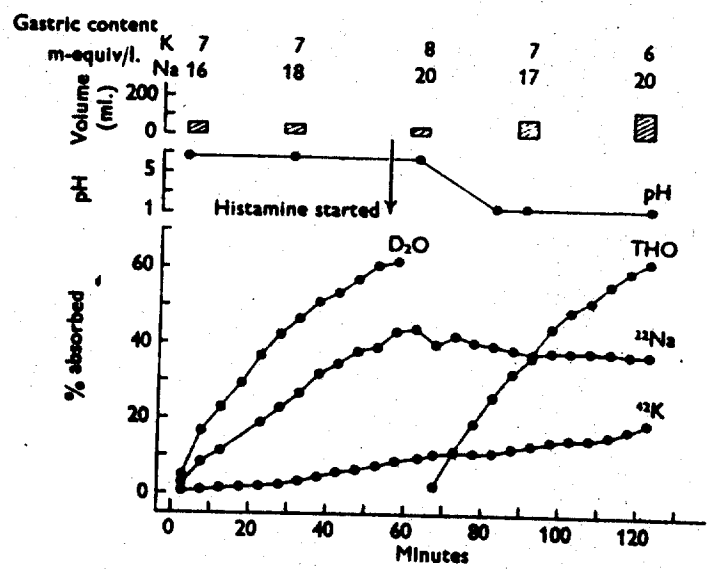

Fig. 11. Simultaneously determined effects on absorption of THO, ^{22}Na, and ^{42}K of acidification of gastric contents from stimulation of secretion of acid.

DISCUSSION

The observations of Cope, Blatt & Ball (1943), Cope, Cohn & Brenizer (1943) and Reitemeier *et al.* (1957) have been confirmed and extended. When acid is added to the gastric contents, whether from intrinsic or extrinsic sources, sodium no longer passes in significant quantities through the mucosa of the corpus or fundus of the stomach into the blood stream. The barrier to the absorption of sodium is not dependent upon the active secretion of acid by the mucosa but upon the ratio of sodium to hydrogen ions in the contents. The results indicate that sodium ions leave the gastric contents to enter the blood stream via a mechanism which is influenced by H ions in such a way that the two ions compete for its control. Hydrogen ions are greatly favoured however. Sodium ions must outnumber those of hydrogen by a factor of at least 10 before they gain access to the mechanism. The absorption of water and potassium is independent of this competition. Their rates of passage from gastric contents to blood stream differ greatly from one another and from the rate of absorption of sodium (Fig. 9); and therefore it seems most likely that they move through the gastric mucosa by pathways which are independent of one another and of the pathway of sodium.

The barrier to the passage of sodium ions offered by the gastric mucosa is

probably related to its ability to secrete hydrogen ions. Bond & Hunt (1956) have demonstrated in cats that elimination of the barrier by fluoride is associated with a reduced secretion of hydrogen ions.

SUMMARY

1. Isotopically labelled water, sodium and potassium were placed in the stomachs of fasting anaesthetized dogs through tubing introduced via the duodenum and the absorption of the isotopes from the gastric contents into the blood was determined.

2. When the contents of the stomach were neutral, sodium was absorbed slowly at a mean rate of 0·44 %/min, but when the reaction of the gastric contents was acid, sodium no longer passed in significant quantities through the mucosa into the blood stream.

3. A relationship was found between the percentage of sodium absorbed from the stomach and the ratio of sodium to hydrogen ions in the contents.

4. The acidification of the gastric contents by intrinsic or extrinsic hydrochloric acid had no effect upon the rate of absorption of water (mean 1·65 %/min) or on the rate of absorption of potassium (mean 0·11 %/min).

5. The results indicate that sodium and hydrogen ions compete for control of the mechanism in the gastric mucosa by which sodium leaves the contents, but water and potassium move from the contents to the blood stream by mechanisms which are independent of one another and of that used by sodium.

This investigation was supported in part by grant no. A-2827 from the Institute of Arthritis and Metabolic Diseases, National Institutes of Health, United States Public Health Service.

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HCl
Unid. French pub. pages 1140-1141

Slow intra-duodenal injection of dilute hydrochloric acid in man, and its influence on glycemia

Note by L. Deloyers and A. Suprez, presented by J. La Barre

Freud and Saadi Nazim (1) in 1926 showed that an intraduodenal injection of dilute hydrochloric acid entrained, at the same time, an increase in the external secretion of the pancreas and a drop in glycemia. Zunz and LaBarre (2), in 1928, noted that this hypoglycemia consecutive to the introduction of the acid, was the consequence of exaggerated insulin secretion. Finally, La Barre and Houssa (3) reported that in dogs with exocrine portion of the pancreas in a state of degenerescence following ligature of the excretion ducts, the intraduodenal administration of acid causes further hypoglycemia, attributable to a post-secreting hyperfunction of the islands of Langerhans.

In the present study, we attempted to demonstrate, in man, the hypoglycemic effects of the introduodenal injection of acid.

We selected 10 patients with serious gastro-intestinal diseases or histories, two patients gastrectomized for gastric ulcer according to the Reichel-Polya technique, and one patient formerly gastro-enterostomized.

By means of an Einhorn probe we injected into the duodenum of these subjects, 250 cc of 0.6% HCl in 20 minutes, i.e. drop by drop, quickly. By radioscopic control we insured the positioning of the probe at the start of the second (portion ?) of the duodenum. Blood samples designed to determine glycemic (variations ?) were made in the normal state, then 30 minutes, 1 hour and (?) after the end of the intraduodenal installation of hydrochloric acid.

For the 22 subjects envisaged, we observed a manifest hypoglycemia in 85% of the cases. In 4 cases the drop in blood sugar reached 10 to 25%. In 10 cases it was 25 to 45%,and in the 4 remaining cases, above 45%. The maximum hypoglycemia (supervened ?) in the majority of the patients one hour after duodenal acidification (15 cases). Note too that this proof was clearly positive in the patients who had previously undergone a gastrectomy or a gastro-enterostomy. In 4 cases out of 22, the introduction of acid in the (duodenum ?) produced no appreciable glycemic alterations.

Consequently, just as in the dog, the intraduodenal injections of hydrochloric acid entrain, in man, in 82% of the cases, an often marked drop in the glycemia, which reached its maximum in the hour following the end of the acidification. These results tend to prove that duodenal secretions play an

important role among the factors which the organism puts into play to compensate for any glycemic rise resulting from the ingestion of meals containing (glucidic ?) substances.

(Laboratory of Pharmacodynamics and Therapeutics, University of Brussels.)

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(Translated by Carl Demrick Associates,Inc/GCT/t)

HCl

French Pub. pages 1140-1141

Slow Intraduodenal injection of dilute HCl into man, and its influence on glycemia

By Deloyers and Duprez

22 human patients injected via Einhorn probe in the duodenum with 250 cc of 0.65% HCl by rapid drip.

Just as in the dog, intraduodenal injections of HCl entrain in man, in 82% of the case, a sudden marked drop of glycemia, reaching maximum in the hour after the end of the acidification.

Deloyers-'46
Bib No. 84

Compt. Rend. Soc. De Biol. 140:1140-1141.1946.

L'INJECTION INTRADUODÉNALE LENTE D'ACIDE CHLORHYDRIQUE DILUÉ CHEZ L'HOMME ET SON INFLUENCE SUR LA GLYCÉMIE.

Note de L. DELOYERS et A. DUPREZ, présentée par J. LA BARRE.

Freud et Saadi Nazim (1) montraient en 1926 qu'une injection intraduodénale d'acide chlorhydrique dilué entraîne en même temps une augmentation de la sécrétion externe du pancréas et une baisse de la glycémie. Zunz et La Barre (2) notaient, en 1928, que cette hypoglycémie consécutive à l'introduction d'acide était la conséquence d'une exagération de l'insulinosécrétion. Enfin La Barre et Houssa (3) signalaient que, chez des chiens à portion exocrine du pancréas en état de dégénérescence à la suite de la ligature des canaux excréteurs, l'administration intraduodénale d'acide détermine encore de l'hypoglycémie attribuable à un hyperfonctionnement postsécrétinique des îlots de Langerhans.

Dans la présente étude, nous avons cherché à mettre en évidence chez l'homme les effets hypoglycémiques de l'injection intraduodénale d'acide.

Nous avons choisi 19 malades indemnes d'affection ou d'antécédents gastro-intestinaux notables, deux malades gastrectomisés pour ulcère gastrique d'après la technique de Reichel-Polya et un malade anciennement gastro-entérostomisé.

Au moyen d'une sonde de Einhorn, nous avons injecté dans le duodénum de ces 22 sujets 250 cm³ de HCl à 0,6 p. 100 en 20 min., c. à d. en goutte à goutte rapide. Par un contrôle radioscopique, nous nous sommes assurés de la position de la sonde au début de la seconde portion du duodénum. Des prélèvements de sang destinés à déterminer les variations

(5) K. Bialaszewicz. *Protoplasma*, 1929, *t. 6*, p. 1.
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(3) La Barre et Houssa. *C. R. Soc. Biol.*, 1932, *t. 110*, p. 596.

glycémiques ont été faits à l'état normal, puis 30 min., 1 h. et 1 h. après la fin de l'instillation intraduodénale d'acide chlorhydrique dilué.

Pour les 22 sujets envisagés, nous avons observé une hypoglycémie manifeste dans 82 p. 100 des cas. Dans 4 cas, la chute du sucre sanguin atteint 10 à 25 p. 100. Dans 10 cas elle était de 25 à 45 p. 100 et dans les 4 cas restants, supérieure à 45 p. 100. Le maximum d'hypoglycémie survint chez la majorité des patients 1 h. après l'acidification duodénale (15 cas). Notons également que cette épreuve fut nettement positive chez les malades qui avaient préalablement subi une gastrectomie ou une gastro-entérostomie. Dans 4 cas sur 22, l'introduction d'acide dans le duodénum n'a pas produit de modifications glycémiques appréciables.

En conséquence, tout comme chez le chien, les injections intraduodénales d'acide chlorhydrique entraînent chez l'homme dans 82 p. 100 des cas une chute souvent marquée de la glycémie qui atteint son maximum dans l'heure qui suit la fin de l'acifidication. Ces résultats tendent à prouver que les sécrétions duodénales jouent un rôle important parmi les facteurs que l'organisme met en jeu pour compenser toute élévation glycémique résultant de l'ingestion de repas contenant des substances acidiques.

(Laboratoire de Pharmacodynamie et de Thérapeutique, Université de Bruxelles.)

Dostal-'73
Bib no. 91

Folia Morphol(Prague). 21(1):97-101. 1973.

No.1 FOLIA MORPHOLOGICA Vol. XXI (1973)

Effect of Some Nonspecific Factors Accompanying Intraamniotic Injection in Mouse Foetus

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Received for publication September 14, 1972

If a pregnant female is given a pharmacological preparation, its teratogenic properties, as well as affecting the embryo directly, can be mediated by the maternal organism or the site of maternal-foetal exchange (Beck et al. 1967). The first question in the pathogenesis of experimentally induced malformation, therefore, is the part of the maternal-foetal complex in which the teratogenic situation develops. One convenient way of resolving this question is to isolate the foetus from the maternal-foetal complex and to study separately the direct effect of a substance on the foetus and its effect when it acts through the whole female-foetus complex. This quickly shows us whether the substance acts on the foetus directly, or whether its effect is mediated by the other parts of the maternal-foetal complex. If the active factor is a metabolite, this method allows the activity of various metabolites to be tested and permits us to verify whether the test substance is metabolized in the foetus and the maternal organism in the same way. If the substance (or a metabolite) acts on the foetus directly, administration of the teratogen to the isolated foetus abolishes variability of the intensity of the teratogenic impulse caused by its modification in different maternal organisms of the same or a different strain or species of laboratory animals.

When choosing the method of isolating the foetus from the maternal-foetal complex, physiological maternal-foetal relationships must be respected, as demonstrated by the different results of metabolic studies done in vitro and in utero (Solomon and Friesen 1968). When studying the development of certain foetal systems, spatial relationships with structures in the immediate vicinity must likewise be maintained. For instance, we can study in vitro the effect of a drug on proliferation of the cell population in extirpated palatine processes, or the terminal phase of development of the secondary palate (union of the palatine processes in the midline), but not the effect of a teratogen on the relationship of the tongue and the growing palatine processes — so often discussed in association with the development of cleft palate.

Intraamniotic injection meets both the above conditions, i.e. integrity of the foetus and maintenance of physiological maternal-foetal relationships. The teratogenic effect of actual puncture of the amniotic sac (described by Traa-

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ler et al. 1956. Walker 1959. Kreshover 1960 and Poswillo and Roy 1965) can be abolished by employing an adequately sparing method (Dostál 1971). In our laboratory we verified in numerous experiments that 2 μl physiological saline is a harmless vehicle for intraamniotic injection. The present report deals with certain factors (nonspecific from the aspect of the substance administered) which might imitate the specific effect of a drug in intraamniotic injection.

MATERIAL AND METHODS

The experiments were done with random-bred H-Velaz mice. The females were mated over-night and the day when a vaginal plug was found counted as the first day of pregnancy. On the 13th day of gestation, 2 μl distilled water, 0.45% NaCl solution, 10% NaCl solution, 0.001 M NaOH solution (calculated pH = 11) and 0.1 M HCl solution (calculated pH = 1) and 10 μl physiological saline were administered intraamniotically to groups of foetuses from at least five females (for the method see Dostál 1971). In every case only the foetuses in the right uterine horns were treated, the untreated foetuses in the contralateral horns acting as the controls. The results were read on the 16th day of gestation. Foetal mortality and the incidence of cleft palate in surviving embryos were studied.

RESULTS

The numbers of experimental females, treated foetuses, dead foetuses and foetuses with cleft palate (CP) in the individual experimental groups are shown in Tab. 1. Tab. 2 gives the numbers of both the experimental and the control foetuses. Both tables include a group given 2 μl physiological saline (Dostál 1971), which we used as the standard.

Table 1. H-Velaz mice. Effect of different intraamniotic treatments on 13th day of gestation

Treatment	Number of females	Foetuses				
		Total	Dead	%	CP	%
2 μl:						
10% NaCl	6	20	7	35	0	0
0.9% NaCl	12	41	4	9.8	0	0
0.45% NaCl	5	24	2	8.3	1	4.2
H_2O (distilled)	6	17	3	17.6	0	0
0.1 M HCl	5	17	5	29.4	1	5.8
0.001 M NaOH	7	24	11	45.8	0	0
10 μl:						
0.9% NaCl	5	18	1	5.6	0	0

Cleft palate was found in only two foetuses — one given 0.1 M HCl solution and one given 0.45% NaCl solution (1.6% of the total). The frequency of cleft palate in the controls was practically the same (2.9% of the surviving foetuses).

Even without statistical demonstration it was evident that foetal mortality after treatment with 2 μl 0.45% NaCl (8.3%), distilled water (17.6%) and 10 μl physiological saline (5.6%) did not differ significantly from mortality after the injection of 2 μl physiological saline (9.8%), but mortality after 2 μl 10% NaCl (35%), 0.1 M HCl (29.4%) and 0.001 M NaOH solution (45.8%) indicated that these substances might have a lethal effect. In statistical evaluation of mortality differences between the individual groups of experimental foetuses, however, we first of all needed to determine whether we

Table 2. H-Velas mice. Effect of different intraamniotic treatments on 13th day of gestation. Control foetuses from left uterine horns of experimental females

Treatment	Treated foetuses			Control foetuses		
	Normal	Dead	CP	Normal	Dead	CP
2 µl:						
H_2O (distilled)	14	3	0	29	3	0
0.45% NaCl	21	2	1	18	0	0
0.9% NaCl	37	4	0	37	1	1
10% NaCl	13	7	0	17	5	1
0.1 M HCl	11	5	1	17	0	2
0.001 M NaOH	13	11	0	32	1	0
10 µl:						
0.9% NaCl	17	1	0	19	1	1

were actually dealing with the specific effect of the given substance and not with the effect of some other, random factor occurring in only some of the experimental groups. With each experimental foetus we took the corresponding control foetus with the same position in the contralateral horn and in each of these matched pairs we determined the presence or absence of the given marker. We differentiated three types of pairs: 1. only the experimental foetus was dead, 2. only the control foetus was dead, 3. both foetuses were either dead or alive. The numbers of the individual types of pairs in the various experimental groups are given in Tab. 3. We based this procedure on the assumption that, while only the experimental foetus would be affected by the direct lethal effect of the test substance, the action of all other concomitant factors on both members of the pair would be equal. As seen from Tab. 3, however, this was not so, as from a total 135 pairs, isolated death of the control foetus occurred in only four cases, while the experimental foetus died in 28 pairs. This is not, pro se, necessarily decisive, as the injection of 2 µl physiological saline is also lethal in a given percentage of cases, but if that were the cause of death in all the 28 pairs we should expect mortality to be the same in all the experimental groups. We tested the null hypothesis, i.e. that mortality among the experimental foetuses in the various groups does not differ, by the χ^2 test in the form of a 2×C contigency table (Snedecor and Cochran 1968) and rejected it ($\chi^2 = 15.88$ for 5 degrees

Table 3. H-Velas mice. Effect on mortality of different intraamniotic treatments on 13th day of gestation. The matched sample consists of a matched experimental and control foetus. + = only the experimental foetus dead, 8 = both the experimental and the control foetus dead or alive, — = only the control foetus dead.

Treatment 2 µl	+	Matched samples 8	—
H_2O (distilled)	3	15	2
0.45% NaCl	1	13	0
0.9% NaCl	5	31	1
10% NaCl	4	12	1
0.1 M HCl	5	10	0
0.001 M NaOH	10	14	0
Total	28	103	4

of freedom). We then tested mortality in each experimental group against the standard (mortality after 2 μl physiological saline) by the χ^2 test in the form of a 2×2 contigency table. Mortality was statistically significantly higher after the injection of 2 μl 0.1 M HCl ($\chi^2 = 3.84$) and 0.0001 M NaOH solution ($\chi^2 = 7.29$).

In can thus be concluded that the intraamniotic injection of 2 μl distilled water, 0.45% NaCl and 10% NaCl solution and 10 μl physiological saline does not have a greater teratogenic or lethal effect on the 13-day mouse foetus than the injection of 2 μl physiological saline. The injection of 2 μl 0.1 M HCl and 0.001 M NaOH solution was not teratogenic, but had a demonstrable lethal effect.

DISCUSSION

In the discussion we shall evaluate the practical significance of our observation. When verifying the possibility of intraamniotic injection we used different administration schemes and different saline volumes (up to 20 μl). Since we found CP in some experimental groups (in some cases the incidence was as high as 10%) we cut the duration of the operation and reduced the injection volume to 2 μl. Related to the amount of mouse foetus amniotic fluid (Mc Cafferty 1965), the injection of 2 μl between the 12th and 15 th day of gestation represents a volume change of not more than 5%. We realized that this was not necessarily the cause of the decrease in the CP incidence after the injection of saline. In these very first experiments, other factors also had to be taken into account, e.g. the injection technique and the symetry of the tip of the pipette. Later, therefore, when we tested the intraamniotic injection of substances which could not be dissolved in the required dose in a 2 μl volume, we returned to the question of volume and found that the mouse foetus could be injected with 10 μl saline from the 13th day of gestation onwards. In our opinion, this amount of solvent is adequate in the majority of cases. It is true that abortion was more frequent in the females in this group, but the experimental groups are too small to allow the formation of any conclusions in this respect. Abortion is also observed in mice after the administration of 2 μl. For instance, 21% of the females aborted afer the intraamniotic injection of 6-azauridine in 2 μl saline. In this respect the rat is a more satisfactory animal, as when 6-azauridine was administered to the foetuses of the same number of female rats (62), not a single animal aborted (Dostál and Jelínek, to be published).

The results of the intraamniotic injection of hypotonic and hypertonic saline show that the osmotic pressure of 2 μl solution, in relation to the homoeostatic possibilities of the foetus or the amniotic fluid, is negligible in this phase of development.

The injection of a solution with a low and a high pH likewise did not cause malformation of the foetus, but was followed by a significant increase in mortality. If we are going to take foetal mortality as one of the criteria of the teratogenic effect of a test substance, we must therefore pay attention to the pH of the intraamniotically injected solution. If the pH cannot be modified, the influence of a solvent with the same pH as the injected substance must be controlled simultaneously. It would no doubt be useful to elucidate the mechanism of the lethal effect of an acid or alkaline pH, but, as mentioned in the introduction, our main aim im the present study was to exclude the possible teratogenic effect of the given physical factors.

SUMMARY

We determined the possible teratogenic effect of physical factors participating in the intraamniotic injection of drugs by administering 2 μl distilled water, 0.45% NaCl, 10% NaCl, 0.001 M NaOH and 0.1 M HCl solution and 10 μl saline intraamniotically to random-bred H-Velaz mouse foetuses on the 13th day of gestation. No teratogenic effect was observed in the surviving foetuses in any of the experimental groups. Mortality after the injection of 2 μl of hypotonic and hypertonic saline and 10 μl saline was likewise the same as affter 2 μl saline. The injection of 2 μl 0.001 M NaOH and 0,1 M HCl solution had a significantly greater lethal effect, however. This finding makes control of the pH of intramniotically administered solutions necessary.

Acknowledgments

The author would like to take this opportunity of thanking Dr. Z. Rychter of the Department of Histology, Faculty of Medicine, Charles University, Prague, for his help with the statistical elaboration of the results.

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Dyer-'46
Bib No. 99

J. of the National Cancer Inst. 7(2):67-70. 1946.

EFFECT OF ADMINISTRATION OF HOT WATER, ACIDS, ALKALI, MECHOLYL CHLORIDE, OR ATROPINE SULFATE UPON THE GASTRIC MUCOSA OF MICE [1]

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INTRODUCTION

The papers (*1*) that were presented at a conference on gastric cancer [2] in 1940, supplemented by those (*2*) from a conference [3] held in 1944, cover the literature adequately on both clinical and experimental gastric cancer. The high incidence of gastric cancer in man, its diagnosis, treatment, etiology, and the question of a genetic influence were discussed in detail. No attempt is made at this time, therefore, to review the literature.

Voegtlin (*3*) emphasized the value of the production of a disease under well-defined experimental conditions as an important contribution to an understanding of the process of the disease. At the present time, no confirmed report has been found of the production of gastric adenocarcinoma in animals with the oral administration of any agent. Stewart (*4*) induced neoplasms of the forestomach of mice with the oral administration of methylcholanthrene or 1, 2, 5, 6-dibenzanthracene. Stewart and Lorenz (*5*) succeeded in producing gastric adenocarcinoma in several strains of mice by the injection of emulsions of these two carcinogens directly into the wall of the glandular portion of the stomach. Production of adenocarcinomas in the stomachs of mice by a combination of selective breeding and parenteral injections of methylcholanthrene was recently reported by Strong (*6*).

The etiology of gastric cancer, however, is not known. The present investigation was undertaken with the hope of producing cancers or precancerous lesions in the gastric mucosa of mice by the repeated administration of tissue irritants and of agents which had been shown to modify the secretion of gastric juice. In addition to this treatment, some groups of animals were given an emulsion of 1, 2, 5, 6-dibenzanthracene repeatedly by esophageal intubation (*7*).

The various agents failed to produce chronic gastric lesions under the conditions of the experiment. The report is therefore presented with a minimum of detail.

MATERIALS AND EXPERIMENTAL PROCEDURE

Inbred mice of strains A, C3H, and I, and hybrid LA mice (strain L mice crossed with mice of strain A) of the F_1, F_2, and F_3 generations were selected for the experiment. Both males and females were used, except in the case of mice of strain C3H, of which only males were selected because of the high incidence of spontaneous mammary tumors in the females. The mice were separated according to sex and were housed in metal, raised-bottom cages in groups of five.

Pellets similar in composition to diet 77 (*8*) were fed ad libitum, except on treatment days when the food was removed from all cages approximately 4 hours before the injections and was returned within the hour following treatment. When the material was administered in the drinking water, the food was removed from these cages also for the same periods as those in which the injected mice fasted. Water in bottles with special outlet tubes was available at all times.

The agents administered were hot distilled water, hydrochloric acid, lactic acid, sodium hydroxide, mecholyl chloride (acetyl-β-methylcholine chloride), and atropine sulfate. Groups of mice from each of the strains were evenly distributed with regard to age and sex and then subjected to treatment with each agent. The mice from those groups that also received repeated injections of an emulsion of 1,2,5,6-dibenzanthracene were matched with mice that received a control emulsion of mineral oil and sodium alginate supplementary to the specific agent.

The carcinogenic hydrocarbon was emulsified by the gradual addition of a solution of 1,2,5,6-dibenzanthracene in hot mineral oil to a hot aqueous solution of sodium alginate. A mechanical stirrer was used during the addition of the hydrocarbon solution, and the dissolved alginate was kept in a water bath at a temperature below boiling. The final emulsion contained, per cubic centimeter, approximately 2 mg. of dibenzanthracene and 8 mg. of sodium alginate.

During the course of the treatments, the mice suffered such a loss of appetite and of body weight that the regimen as originally adopted had to be changed. The dosage was decreased; the periods of treatment were shortened; the intervals between treatments were increased; and whenever the majority of the mice showed loss of weight or other unfavorable symptoms, the injections were discontinued temporarily. During the first 5 months of the experiment injections were made 5 times weekly from Monday through Friday, except those with mecholyl and atropine sulfate, which were administered 10 times weekly (twice a day on each of the 5 days). The dibenzanthracene

[1] Received for publication August 23, 1946.
[2] Under the auspices of the National Advisory Cancer Council, held at the National Institute of Health, Bethesda, Md., on October 11 and 12, 1940.
[3] Under the auspices of the Committee on Cooperation in Cancer Research, National Advisory Cancer Council, United States Public Health Service, held at The Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York City, on November 11, 1944.

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and control emulsions were begun later. At this early period, because of the great mortality among the treated mice, additional animals were started in each group, and the number of injections was decreased to 3 a week on alternate days. When the dibenzanthracene and control emulsions were given during a later period, they were administered once a week. Lactic acid and atropine sulfate were furnished continuously in the drinking water except during short periods when great loss of weight was observed and the mice were given drinking water without the supplements.

For the treatment with hot water care was taken to cool the needle by immersion in cold water before inserting it in the mouth of the animal.

The effect of subcutaneous injections of mecholyl into mice upon the secretion of gastric juice was investigated before the repeated injections were begun. The procedure described by Roe and Dyer (*9*) was followed except that urethane was used to anesthetize the mice and the peptic activity was measured by a modification (*10*) of the method of Anson. An attempt was made to prolong the action of mecholyl chloride by incorporating it into a beeswax-liquid petrolatum emulsion and injecting it intramuscularly in amounts up to 2.0 mg. of mecholyl per mouse. Profuse salivation and bulging of the eyes and occasional gastric hemorrhages resulted, but the action of the mecholyl was not prolonged significantly. After three such injections into the experimental mice, the subcutaneous injections of aqueous solutions of the gastric stimulant were resumed.

After 2 months of subcutaneous administration of atropine sulfate, intramuscular injections of a beeswax-liquid petrolatum emulsion of this substance were tried. Abscesses appeared at the sites of injection, so that the subcutaneous injections of the aqueous solution were resumed after 10 days. When the number of injections of the various agents was reduced to three a week, the surviving mice in this group and the new mice added to the group were given atropine sulfate in their drinking water instead of subcutaneously.

Preliminary tests with esophageal intubations of the alginate-mineral oil emulsion to mice that were killed at intervals of 0.5, 2.0, and 6.0 hours following the injection demonstrated the persistence of a white gel in the stomach. The gel adhered to the gastric mucosa and frequently appeared to form a plug in the pyloric region of the stomach. Supplementary injections of hydrochloric acid or of hot water had no observable effect upon the persistence of the emulsion in the stomach; sodium hydroxide changed the white gel of the injected emulsion to a yellowish, mucilaginous fluid which was found to remain in the stomach for some hours.

The loss of mice was so great during the first year of the investigation that it was decided thereafter to discontinue treatment of all mice that had been treated for as long as 6 months (65 to 75 injections) and to keep the surviving animals under observation until they began to show signs of abnormality, or loss of weight, or had reached the age of 12 to 15 months.

The dosages of the agents and the numbers of injections are summarized in table 1.

TABLE 1.—*Summary of treatment of mice*[1]

Agent	Concentration[2]	Number of injections per mouse[3]		Number of mice		Age of Mice at—				Final weights of mice		Appearance of stomachs at necropsy			
						First treatment		Necropsy				Gross		Microscopic	
		Range	Average	Died	Killed	Range	Average	Range	Average	Range	Average	Normal	Hemorrhagic	Normal	Pathologic[4]
						Months	*Months*	*Months*	*Months*	*Grams*	*Grams*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Water, approximately 60° C.		15–141	57	34	28	1–11	4	3–22	11	11–41	23	92	8	93	6
Control emulsion		4–23	13			1–13	6								
Water, approximately 60° C., and		11–120	67	14	18	1–6	3	3–17	11	10–36	24	94	6	72	0
Dibenzanthracene emulsion.	2 mg. per cc.	3–16	11			1–8	5								
Water, room temperature		16–131	62	20	14	1–5	4	7–18	12	17–34	26	93	7	93	7
Control emulsion		3–23	13			1–10	5								
Water, room temperature, and		27–138	75	15	17	1–6	2	5–18	11	15–46	29	97	3	82	12
Dibenzanthracene emulsion.	2 mg. per cc.	2–17	13			1–6	3								
Hydrochloric acid	1.0 to 0.25N	10–92	41	35	23	1–7	3	2–18	9	10–45	24	88	12	65	4
Control emulsion		3–21	12			1–10	4								
Hydrochloric acid, and	1.0 to 0.25N	10–99	54	18	22	1–7	3	4–17	11	13–52	25	93	7	86	0
Dibenzanthracene emulsion.	2 mg. per cc.	2–24	12			1–6	3								

See footnotes at end of table.

TABLE 1.—*Summary of treatment of mice*[1]—Continued

Agent	Concentration [2]	Number of injections per mouse [3]		Number of mice		Age of Mice at—				Final weights of mice		Appearance of stomachs at necropsy			
						First treatment		Necropsy				Gross		Microscopic	
		Range	Average	Died	Killed	Range	Average	Range	Average	Range	Average	Normal	Hemorrhagic	Normal	Pathologic [4]
						Months	*Months*	*Months*	*Months*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*
Sodium hydroxide	0.5 to 0.1N	20-132	51	19	17	1-8	3	3-18	10	17-37	28	90	10	70	12
Control emulsion		1-24	13			1-10	4								
Sodium hydroxide, and Dibenzanthracene emulsion.	0.5 to 0.1N 2 mg. per cc.	12-65 1-16	38 10	23	11	1-8 1-6	3 4	4-19	9	14-42	24	89	11	82	9
Mecholyl chloride	0.05 to 0.5 mg. per cc.	56-306	151	19	15	1-5	2	5-17	9	13-38	25	91	9	73	0
Control emulsion		1-22	12			1-5	4								
Mecholyl chloride, and Dibenzanthracene emulsion.	0.05 to 0.5 mg. per cc. 2 mg. per cc.	62-286 3-22	168 13	15	11	1-5 2-5	2 4	5-15	11	17-37	26	89	11	91	0
Atropine sulfate, s. c., or Atropine sulfate in drinking water.	0.1 to 1.0 mg. per cc. 0.25 mg. per cc.	50-266 21-100	216 69	23	12	1-10 1-10	2 5	2-17	9	15-43	26	83	17	92	0
Control emulsion		4-18	11												
Atropine sulfate, s. c., or Atropine sulfate in drinking water, and Dibenzanthracene emulsion.	0.1 to 1.0 mg. per cc. 0.25 mg. per cc. 2 mg. per cc.	25-265 53-100 5-17	200 86 12	17	14	1-7 1-7	2 4	6-20	11	15-40	25	87	13	100	0
Lactic acid	0.2 to 0.1N	31-153	91	18	17	1-9	3	8-16	13	15-38	25	91	9	82	12
Control emulsion		7-28	15			1-9	4								
Lactic acid, and Dibenzanthracene emulsion.	0.2 to 0.1N 2 mg. per cc.	45-160 8-32	93 14	5	13	1-6 1-6	3 4	5-15	11	13-37	24	78	22	100	0

[1] Mecholyl chloride was administered subcutaneously; atropine sulfate, subcutaneously and in drinking water; lactic acid in drinking water; and all other agents by esophageal intubation.
[2] The volumes administered were 0.01 cc. of agent per gram of body weight for all esophageal intubations and subcutaneous injections, and ad libitum when agent was administered in the drinking water.
[3] When agent was administered in drinking water the values are for days of treatment per mouse.
[4] Miscellaneous pathologic changes other than typical lesions in mice of strain 1.

The mice were weighed biweekly during the period of treatment and once a month between the last injection and the time of killing. Many of the mice died during the night, and post mortem changes were too advanced for necropsy. Where post mortem changes were not too great, gross examination was made of each animal that died, special care being exercised in the examination of the gastrointestinal tract. The remaining mice were killed as stated previously. They were necropsied, and histologic studies were made.

In the routine examination of mice that were killed at an early date, the stomach was removed at once, quickly examined for gross lesions, cut into three longitudinal strips, and fixed in absolute alcohol for Mayer's mucicarmine stain, Regaud's solution for Bowie's pepsinogen stain, and Zenker-formol for routine hematoxylin-eosin stain. The rest of the organs were then examined, and those showing gross abnormalities were taken for routine hematoxylin-eosin stain. Later in the experiment, special stains on the stomach were usually omitted, but the routine hematoxylin-eosin stain was made of the stomachs of all the experimental mice that were killed, and the special stains and the hematoxylin-eosin stains were used for histologic studies of the stomachs of 15 untreated control mice of strains A, C3H, and 1, and LA hybrids.

RESULTS

Table 1 summarizes the experiments and the findings with regard to the stomachs of all the mice that were necropsied. In the case of the animals that died during treatment, the only abnormality observed in any of the stomachs of any of the groups was hemorrhage. Petechial hemorrhages when present were invariably located in the glandular portion of the gastric mucosa and extended over a very small area. From preliminary studies of the acute toxic effects of the various agents on mice, it seems likely that the stomach hemorrhages were caused by the acute, irritating effects of the agents injected. Hemorrhages were observed in small areas of the intestinal tracts of 9 percent of the treated mice and were found in all groups.

No significant difference in the amount of mucin

or pepsinogen in the gastric mucosa could be detected in any of the groups of experimental or control animals, although occasional mice showed a considerable variation for which no cause was found. Positive mucin staining is found characteristically in a few glands near the limiting ridge (vestiges of a cardiac portion in the mouse), on the surface of the gastric gland or fundic portion, and in cells located at the base of the pyloric glands. The mucicarmine stain did not outline so distinct a layer of mucous cells at the necks of gastric glands as is usually encountered in the rat. Stomachs of strain I mice were of particular interest, since these special staining procedures disclosed a disarrangement of cell types and showed that mucous staining cells were present in the fundic glands before the typical strain I lesion, as described by Stewart and Andervont (*11*), had fully developed.

Except for those of the strain I animals, the stomachs examined were remarkably normal. A few miscellaneous pathologic changes were recorded which did not appear significant. Only 1 mouse, a hybrid LA female, which had received 92 injections of hydrochloric acid and 11 injections of dibenzanthracene over a period of 6.5 months and was killed at the age of 8.5 months, showed an inflammatory reaction about the base of the glands, and some hyperplastic cells. Another hybrid animal treated with atropine sulfate and dibenzanthracene developed an epidermoid carcinoma of the forestomach. Stomachs of the strain I animals showed the changes typical for this strain, which did not seem to be modified by treatment.

Numerous pathologic changes were found in other organs examined; but none of them could be ascribed to any specific experimental procedure, since they occur commonly in any groups of old experimental mice. Lesions in the lungs were very frequent, especially in animals subjected to esophageal injections. These pulmonary changes usually consisted of areas of consolidation caused by a leukocytic exudate or accumulations of large vacuolated cells in the alveoli, bronchitis, peribronchial leukocytic infiltrations, and areas of atelectasis. These pulmonary conditions were probably the most frequent immediate cause of death.

Several types of tumors were found, but they were not significantly more frequent in any particular group. Among them were mammary tumors, leukemias, and lung tumors. Nine of fourteen mice that developed tumors had received dibenzanthracene.

SUMMARY

Repeated direct alimentary administration of hot water, hydrochloric acid, sodium hydroxide, lactic acid, or atropine sulfate, with or without an emulsion of 1, 2, 5, 6-dibenzanthracene, failed to produce cancer or precancerous lesions in the gastric mucosa of mice of strains A, I, and C3H, and in hybrid LA mice (strain L crossed with mice of strain A).

Repeated parenteral injections of mecholyl chloride or of atropine sulfate, with and without direct alimentary administration of 1, 2, 5, 6-dibenzanthracene, were likewise ineffective in producing any specific pathologic changes in the gastric mucosa of mice.

That the stomachs showed so little injury following very drastic treatment is evidence of the remarkable resistance and recuperative power of the normal gastric mucosa.

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J. Indiana M. A. 27:253-254. 1934.

Ellison-'34
Bib no. 104

PALINESTHESIA*

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Since the time of Wells, Morton and Simpson, the profession has been able to induce general anesthesia and has subsequently spent much effort in discovering new agents and methods for this purpose. Thus far, however, no means has been available for quickly reversing the process, terminating a general anesthetic. This article will describe such a method. The term "palinesthesia" is proposed as being descriptive of this phenomenon.

Our attention has been drawn through personal communications to the fact that aqueous solutions of hydrochloric acid, when injected into the blood stream of experimental animals in profound anesthesia, promptly terminate anesthesia.

Moorman,[1] in an effort to save overanesthetized animals, conceived the idea that anesthesia tended to elevate the Ph of the blood. It was reasoned that introduction of an acid into the blood stream would reverse this process and effect the state of anesthesia. Applying this idea, Moorman discovered that profoundly anesthetized animals did promptly and completely awaken following the intravenous injection of minute doses of hydrochloric acid. These observations were verified by the Duncan Laboratories of Kansas City, Missouri, and by ourselves. With this method we have brought about resuscitation in rabbits etherized to the point of respiratory cessation.

Studies in the intravenous use of hydrochloric acid and other acids in their effects on respiration have been made by Eddy,[2] and by Gessel, Kreuger, Gorham and Bernthal.[3] They do not report having injected anesthetized animals in their experiments.

Since knowing of the effects of this medication in experimental animals we have been mindful of the possibilities of its use to resuscitate human beings who might have become dangerously overanesthetized. Such an emergency arose at Memorial Hospital, Worcester, Mass., in July, 1933.

The patient had an unusual reaction to a commonly repeated and conservative dose of avertin. No reflection upon this anesthetic agent is implied, since our personal series with it embraces over two thousand cases with gratifying results. The patient, following operation, was found to be progressively approaching a state of anesthesia so profound that his condition was moribund. The pulse was rapid and weak and the tidal volume was practically nil. He was obviously in extremis and heroic measures were imperative. At this point an aqueous solution of hydrochloric acid was injected intravenously. This injection was started at 10:15 a. m., and during the ensuing twenty minute period 5.4 cc. of a one per cent solution of hydrochloric acid was given.

Immediately following the injection of the first few drops there was a striking increase in the respiratory excursion. Blood pressure at 10:20 was 100/80. At 10:23 there was motion at the angles of the mouth, and two minutes later the jaw closed and the lips puckered. At 10:30 both hands were in motion. At 10:35 the patient swallowed. At 10:40 he gripped the fingers of an attendant. At 10:45 the patient made an effort to eject the metal air way and gave an incoherent response to question. At this moment a further injection of 3.2 cc. of the same solution was begun and continued over the next five minute period. At 10:50 a foot was in motion. Five minutes later the eye balls were oscillating and the palpebral muscles were functioning. At that time the patient answered questions coherently. From this point the patient remained awake. At 9:00 p.m. the patient complained of wakefulness, a condition quite unusual following so soon after avertin anesthesia.

Repeated laboratory studies of this patient have failed to disclose any untoward effect resulting from this medication.

We believe this is the first use of this drug for this purpose in a human subject.

What transpires when solutions of hydrochloric acid are introduced into the blood stream opens many interesting fields of thought and investigation. How the reversal of loss of consciousness, loss of sensation and loss of motion is effected by the injection of HCL is a matter of immediate interest which awaits further investigation. It appears that the discovery of this use for HCL intravenously to terminate anesthesia may prove to be a part of the solution of the physical chemistry of general anesthesia. What actually happens chemically between HCL and the blood components must be the basis for our speculations.

We quote Wright[4] as follows:

"I. Physico-Chemical Reactions. 1. When any non-volatile acid like lactic, phosphoric, or sulphuric acid enters the blood stream, it at once interacts with the bicarbonate present in the plasma as follows:

$$\text{(i) } HL + NaHCO_3 \rightarrow NaL + H_2CO_3.$$

"In the case of lactic acid, sodium lactate and carbonic acid are formed. The advantage of this reaction to the body is clear. Lactic acid is a strong acid, which means an acid which dissociates freely and gives rise to many H ions, and thus makes any solution in which it is present very acid; it is also a non-volatile acid which is excreted slowly in the urine. By the interaction with sodium bicarbonate it is converted into a neutral salt and H_2CO_3 is liberated. Now H_2CO_3 is a much weaker acid, giving rise to few H ions; furthermore, it is a volatile acid which is readily eliminated in the lungs. Sodium bicarbonate has therefore acted as a buffer substance; it has caused H ions to be

* Presented before the St. Joseph County Medical Society, February 6, 1934.

'mopped up,' fewer are left in the solution, and the reaction of the blood has altered to a much slighter extent than otherwise would have been the case. This is why the term 'alkali reserve' was introduced to describe the bicarbonate of the blood.

"It follows that as long as $NaHCO_3$ is present in the plasma, no acid stronger than carbonic can exist in the blood as such.

"2. But the acid formed in largest amounts in the body is H_2CO_3 itself, which of course cannot be dealt with by $NaHCO_3$. H_2CO_3 is dealt with in the following way:

$$\text{(ii) } H_2CO_3 + NaCl \rightleftarrows NaHCO_3 + HCL.$$
$$\text{(iii) } HCL + KHb \rightarrow KCl + HHb.$$

H_2CO_3 interacts with the sodium chloride of the plasma to form sodium bicarbonate and hydrochloric acid. On the face of it this reaction seems both meaningless and impossible. Instead of the weak acid H_2CO_3, we have formed the stronger acid HCl, so the H ion concentration of the solution ought to rise still higher. Again, a weak acid like H_2CO_3 cannot displace chloride from its union with Na in NaCl. The explanation is this. An equation like ii above can act in either direction if the product of the reaction that would tend to drive the process the opposite way is at once removed. In other words, the equation will go from left to right if the HCl formed is at once removed from the plasma. This actually happens. If blood is exposed to increased tensions of CO_2, the amount of $NaHCO_3$ in the plasma increases and the chloride diminishes in the plasma and increases in the interior of the red blood corpuscles. The HCl resulting from equation ii, as it is formed in minute amounts, migrates rapidly through the corpuscular wall to be dealt with suitably in the red cell. This is the Hamburger phenomenon, or chloride shift."

The production of a considerable quantity of CO_2 immediately following the injection of this acid into the blood stream accounts for the prompt increase of tidal volume.

It is apparent that equations ii and iii of the foregoing quotation that considerable quantities of reduced Hb are also produced following the injection of such acids into the blood stream. What the effect is of suddenly increasing the amount of reduced Hb in the blood stream of an anesthetized animal is open to speculation.

Attention is directed to the fact that in the case of the patient herein reported the 8.6 cc. of one per cent solution of hydrochloric acid represents about 1 minim of the acid. The blood volume of an average individual is approximately 100,000 minims. It is therefore evident that the alteration of the blood, producing palinesthesia, is extremely small and it is probable that lowering of the blood Ph by this minute addition of acid may be the explanation we are seeking.

The revival of patients who are endangered because of overanesthetization, while important in itself, is not the only application for this agent.

Cases of impending death from drowning, severe electric shock, surgical shock, asphyxia from toxic gases and alkelemias might be benefited by its use. It would be assumed that heart function is still present.

Cases of sleepiness, such as occur in long sustained aeroplane flights, offer a possibility for its use. This is suggested by the fact that our patient, who normally would have been sleepy, complained of annoying wakefulness for many hours following the intravenous use of HCl.

Many interesting problems for research are suggested by the results of this new use of HCl.

Among these are:

(a) The intravenous injection of reduced Hb to anesthetized animals.

(b) A study of changes in the PH of the blood, before, during and following general anesthesia, associated with the injection of HCl.

(c) A study of the effects of alkalinization of subjects preceding anesthesia, in view of the information disclosed by the intravenous use of HCl.

(d) Studies in the intravenous use of other acids in anesthetized animals.

From our limited experience with this medication we believe that it may be a life saving measure on occasions suggested above, when the usual procedures have failed.

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HEART DISEASE COMPLICATING PREGNANCY*

DIAGNOSIS AND TREATMENT

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More than ninety per cent of significant heart disease in pregnancy is of the rheumatic type. The usual lesions are mitral stenosis and insufficiency with aortic insufficiency in some cases.

The purpose of this paper is to emphasize certain broad principles regarding heart diseases in pregnancy.

The first of these is that pregnancy itself may cause a fatal overload to a previously damaged heart. It is well recognized that the strain of labor may precipitate heart failure in such cases, but the cardiac overload from pregnancy itself needs emphasis.

* Presented before Indianapolis Medical Society, 1933.

Orvosi Hetilap 90:777-781. 1949.
Published by The Forensic Medicine Institute of the Szeged University of Sciences. Director of the Institute: Dr. Julius I. FARKAS, honorary lecturer.

Fazekas-'49
Bib No. 111

Experimental data on simple compounds used for influencing ovarian functions.

By Dr. Julius I. FAZEKAS.

During the last two decades, the following substances were mainly used, more or less successfully, for replacing or restoring the function of the ovary when its functioning was reduced: the folliculus hormone and the corpus luteus hormone or the gonadotropic hormone of the front lobe of the hypophysis (prolan A, prolan B). Some observed good results after the administration of stilbene derivatives. A few authors found results favorable in this field after X-ray treatment of the overy, others after X-ray treatment of the hypophysis; some found that the results were favorable with a carbohydrate-free ketogenic (meat) diet (LORALEK), etc. Since it is not always possible to restore the ovary function with these methods on the one hand, and since hormone treatment in particular is rather costly on the other hand, it appears desirable to search for a treatment method which would eliminate the disadvantages of the therapeutic methods applied to date.

In my earlier works I disclosed my investigations on the basis of which I showed that when adequate dosages of certain chemical compounds are supplied, the suprarenal glands of various animals become enlarged and the cortical substance function of the same intensifies, as compared to normal functioning. In the course of these tests I observed facts which indicate that it is also possible to act on the ovary function without using any hormones, by applying adequate dosages of simple compounds.

My first findings in this respect were made in 96 tame rabbits, treated with NH_4OH. During these tests, female tame (domestic) rabbits, weighing 2500 to 3500 gr, kept separately , which were sexually mature (aged 8 to 14 months) were given every second day 50 to 60 ccm $\frac{1}{2}$% NH_4OH per os; they were killed after 1 to 3 months. We then found suprarenal gland cortical substance hyperthrophy as well as folliculus maturation in the ovary, hemorrhaged folliculi and the formation of yellow corpuscles, together with a certain degree of enlargement. Our second observations concerned geese; for the purpose of increasing the function of the suprarenal glands, we supplied every second day 50 to 70 ccm $\frac{1}{2}$% NH_4OH through the stomach tube in increasing doses during 4 weeks to 10 geese originating from the same hatching. We gave the same feed, without treatment, to 10 other geese from the same hatching for control purposes. The geese were killed after 4 weeks; numerous nut-size and larger eggs (ovules) were found in the ovaries of the geese treated with NH_4OH, which in each goose formed a total mass having the size of a man's fist. As compared to the above, the eggs (ovules) present in the ovaries of the geese which had received the same feed but without treatment were less than green-pea-size.

We performed additional tests on the basis of these preliminary findings, during which sexually mature (aged 8 to 14 months) female, virgo intacta, separately kept tame rabbits of the same breed (chinchilla) were used; they received the same food. Tame rabbits are especially adequate for tests intended for the artifical stimulation of the ovary function. We know from investigations performed by VAN BENEDEN and HEAPE, as well as from the tests performed by others that in tame rabbits ovulation, or the rupture of the folliculus occurs only after copulation.

It is, however, known that folliculus rupture occurs in tame rabbits after "sterile mating" as well. Activated by the corpus luteum which then forms, characteristic changes occur in the genitalia (enlargement of the womb, decidua formation, enlargement of the teats). This condition is usually defined as "false pregnancy" or "apparent pregnancy". The false pregnancy condition lasts for 13 to 14 days according to ANCEL and BOUEN, and for 16 to 19 days according ti HAMMOND; it lasts for 16 days according to KNAUS; then it regresses as a result of corpus luteum degeneration. To avoid a "false pregnancy", the tame rebbits must be kept separated for at least 1 month before testing of the ovarian function or the condition of the genitalia respectively, is started.

In the course of the tests reported below, we isolated the control rabbits already at the age of 5 months, i.e. 1 month before they reached sexual maturity (6 months), and we kept them separately for 3 to 8 months before using tham for the test. As a matter of course, the separation continued during the test period as well. The purpose of this procedure was to have ovaries of various age available for examination which , while sexually mature, had not yet undergone --for natural reasons--any follicle maturation and rupture, nor corpus luteum formation; we thus could evaluate the effect of the treatment with maximal precision.

For the first test series we used female tame rabbits, with an initial weight of 2500 to 3500 g and aged 8 to 14 months; we assigned them to groups including 5 to 7 animals each (kept separately); we used different compounds for the treatment of each group. Each animal received, in gradually increasing doses, 0,1 to 0,2 g per kg of body weight of the active agent, dissolved in 100 to 150 ccm of drinking water, applied every second day. The duration of the treatment was 5 months, carried out as follows: a period of one week without treatment was inserted in all cases after a treatment period of three weeks. The following compounds were used for the treatment: ammoniumchloride, ammoniumsulfate, ammoniumcarbonate, sodium-ammonium phosphate, ammoniumacetate, ammoniumlactate, calciumchloride, acidum hydrochloricum (25%), acidum lacticum cc, acidum aceticum, sodium dihydrophosphate, ammonium hydrophosphate.

With the applied treatment, the acid base equilibrium of the animals periodically shifts to a lesser extent towards acidity; but no serious acidosis, as currently interpreted, did occur. The animals ate with good appetite and gained weight throughout the (test) period. For control purposes we kept 50 female, virgo intacta tame rabbits of the same age, breed and intial weight likewis separated; they received the same quality and quantity feed.

After 5 months we killed the treated and the control rabbits by means of air embolims; we found that the ovaries of the treated animals, as compared to those of the controls, were more or less enlarged in all cases; on their surface , visible to the unaided eye, numerous enlarged and mature hemorrhaged follicles or corpora lutea were in evidence.

Enlargement of the ovaries of tame rabbits as a consequence of treatment with various compounds.

1 Sor-szám	2 A kezelés módja	3 Az esetek száma	4 A 2 ovarium súlya cg			A 2 ovarium súlygyarapodása középértékben		9 S. d.
			5 minimum	6 maximum	7 középérték	cg	%	
1.	Kontroll	50	15	52	38.66	—	—	—
I.	Ammonium hydroxyd	96	42	150	63.24	24.58	63.57	6.92
2.	Ammonium chlorid	50	53	108	70.82	32.16	88.36	5.82
3.	Ammoniumsulfát	5	68	128	95.50	56.84	147.00	7.63
4.	Ammonium carbonát(l)	7	60	92	80.70	52.04	134.60	6.34
5.	Na.-ammon. phosph.....	7	67	111	95.66	57.00	150.00	9.20
6.	Ammonium acetat.....	6	54	121	87.30	48.64	125.81	8.32
7.	Ammonium lactat	5	68	146	104.66	66.00	173.30	9.24
8.	Calcium chlorid	5	60	146	102.20	63.54	164.35	8.06
9.	Ac. hydrochloricum	5	73	115	93.60	54.94	142.10	7.38
10.	Ac. lacticum	5	64	118	96.40	57.74	150.00	7.65
11.	Ac. aceticum	6	57	102	87.62	48.96	125.80	7.14
12.	Natrium dihydrophosphat ..	5	92	162	126.33	87.67	229.35	11.32
13.	Ammon. hydrophosphat	5	81	132	112.00	73.34	190.47	8.27

Sodium

1) Serial No.
2) Treatment method
3) Number of cases
4) Weight of the 2 ovaries, cg
5) maximum 6) minimum 7) average value
8) Weight increase of the 2 ovaries in average values - cg - %
9) Significance

The data of the table show convincingly that under the effect of treatment with the applied compounds, a considerable increase of the ovary weight occurs, reaching an average value of 63.57 - 229.35%. The fact that the weight increase is significant (S.D is proved by the performed statistical(probability) calculations. The enclosed photograph (No.I) is a good illustration of the volumetrical increase, of the hemorrhaged follicles and of the corpora lutea. On said illustration the last (VIth) line shows the ovaries of control rabbits of the same age and with identical weight, without treatment; in the Ist to Vth line the enlarged ovarie of treated rabbits are demonstrated, with a different compound used for each line.

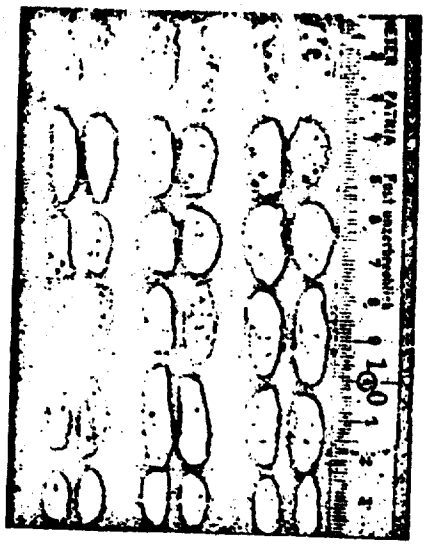

Illustration I. enlargement, folliculus maturation and hemorrhage, corpus luteum formation in the ovaries of virgo intacta tame rabbits treated for 5 months with various compounds.

Line I.	Calcium chloride	treatment	93 cg	127 cg	146 cg
" II.	ammonium acetate	"	83 "	100 "	121 "
" III.	" lactate	"	68 "	100 "	146 "
" IV.	" sulfate	"	85 "	88 "	128 "
" V.	sodium-ammonium-phosphate	"	80 "	90 "	111 "
" VI.	without treatment (control)	"	37 "	40 "	40 "

Histological examination. The sections of the ovaries from the untreated control rabbits show large numbers of primary follicles, crowded closely to each other; between these, maturing but not yet mature, comparatively small follicles are visible (10 to 14 in each section); the interstitial ovary glands (medullary substance) are thus restricted to a comparatively very small area. No completely mature follicles, ready to rupture, are detectable. (Illustration 2.).

On the other hand, in virgo intacta rabbits treated for 5 months and kept separately, the major part of the enlarged ovary consists of interstitial ovary glands (medullary substance); the cells of these glands are conspicuously larger than normal and their number is likewise considerably increased; in their protoplasm, the lipoids multiplied, forming smaller and larger drops, and the nucleus is frequently displaced laterally. In some cases these cells are so rich in lipoids within each spot-like area that they appear very similar to the lutein cells. It is likewise remarkable that the ovaries of some animals show numerous mature follicles, hyperemia, and hemorrhaged follicles (Illustration 3), while the ovaries of other animals show numerous corpora lutea (Illustration 4). Mature and hemorrhaged follicles are found not only on the surface, but also in the deeper layers. The cortical substance of some ovaries narrowed, while others show no narrowing. We did not find folliculus degeneration or ovary cell degeneration in the ovaries of the treated rabbits. This seems to indicate that the applied

treatment does not damage the follicles, or the ovary respectively. The primary follicles and the maturing follicles on the ovaries of the treated rabbits are conspicuously less dense, at a greater distance from each other, as compared to the control ovaries. This can be explained by the fact that on the one hand, the follicles seem to be pushed apart as a result of the enlarged volume and surface of the ovary, thus taking up a larger area; on the other hand, the absolute number of the follicles will of necessity decrease as well, as a consequence of intensified follicle maturation which occurs in the course of the treatment.

Illustration 2. Section of the ovary from an untreated (control) tame rabbit, weight 3000 g, virgo intacta, age 13 months. The total weight of the 2 ovaries is 25 cg.

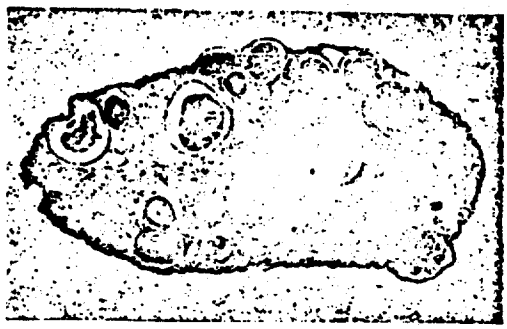

Illustration 3 . Hemorrhaged and mature follicles in the ovary of a 13 months old, virgo intacta rabbit, after treatment with calcium chloride during 5 months. The total weight of the two ovaries is 146 cg. Initial body weight: 3100 g. Body weight at the termination of the treatment: 4200 g. Hemotoxyline-eosin dye.

Illustration 4. Corpora lutea in the ovary of a tame rabbit, virgo intacta, age 13 months, after treatment with ammonium lacticum during 5 months. The total weight of the 2 ovaries: 146 cg. Initial body weight: 3100 g. Body weight at the termination of the treatment: 4700 g. Van Gieson dye.

The above statements lead to the conclusion that the observed ovary enlargement can on the one hand be ascribed to the hyperthrophy of the interstitial overy glands and to the increase of their lipoid content; on the other hand, it can be ascribed to the enlargement, maturation and hemorrhage of the follicles or the corpora lutea formation respectively.

In the course of another test series under the aforementioned test conditions we likewise treated 5 to 7 rabbits (aged 8 to 10 months) with the same compounds (80 to 100 doses); we observed them during 14 to 16 months; they were then killed by means of air embolims and their ovaries were subsequently examined. Substantially similar symptoms were found in the ovaries of these animals as those found after a treatment period of 5 months; I therefore omit a detailed report. It is merely noted that we did not find any degenerative symptoms in the ovaries of these animals.

According to data in literature, an effect causing the maturation of the follicles in the ovary and the formation of corpora lutea was primarily found to be caused by the gonadotropic hormones produced by the front lobe of the hypophysis.

Numerous tests undoubtedly proved that the function of the ovaries is hormonally directed by the front lobe of the hypophysis.

Some authors contend, on the basis of various tests, that the basophylic cells of the hypophyisis produce the hormone which maturates the follicle, while the acidophylic cells produce the hormone which forms the corpora lutea. HERLANT observed a follicle-maturing effect on the occasion of a basophyilic cell transplant which (cells) had multiplied during the SELYE alarm-reaction. After implanting the zone of oxen- and hog-hypophysis which contains basophylic cells into female, sexually mature rats, GIROUD and MARTINET observed a considerable ovary enlargement, follicle maturation and formation of corpora lutea. Following the transplant of an acidophilic cell group, they found that a minor follicle enlargement only developed, while no corpora lutea formed. According to their tests, they attribute the follicle-maturing effect to the basophylic cells of the front lobe of the hypophysis; they believe that the basophylic cells are likewise involved in the formation of corpora lutea.

The formation of corpus luteum was observed by HOHLWEG in young rats and by KLAFTEN, PATAKY and others in ovaries of sexually mature, isolated tame rabbits following the injection of a larger dose of folliculus hormone. They explain this as follows: large quantities of follicle hormore inhibit the hormone (prolan A) production of the front hypophysis lobe, while promoting the production of the corpus luteum-forming hormone (prolan B).

After injecting the urine of a pregnant woman (which was boiled and therefore did not contain any prolan), BAUMANN observed corpus luteum formation in animal ovaries; he attributes this to the effect of the large volume of folliculine in the urine of the pregnant woman, which acts through the hypophysis. HOHLWEG and others share this view. According to the investigations of ZONDEK, CLAUBERG and others the corpus luteum formation is always preceded by a certain degree of follicle maturation, triggered by prolan. AXEL-WESTMANN likewise pointed out that no corpus luteum formation takes place without follicle maturation.

After injecting the urine of men and women kept on a ketogenic diet into infantile white mice, JULESZ observed in the animals follicle maturation and edematous swelling; but no follicle hemorrhage or corpus luteum formation took place. JULESZ explains this phenomenon by stating that the ketosis stimulates the front lobe of the hypophysis to intensified prolan production.

We wish to state additionally that LUIGI caused significant follicle maturation in 'infantile' tame rabbits with electrical stimulation of the hypophysis; FREEGOOD and PINCUS achieved the same in guinea pigs with electrical stimulation of the neck sympaticus.

The results of our own reported tests indicate that it is possible to obtain follicle maturation, follicle hemorrhage and corpus luteum formation in the ovaries of tame rabbits without hormone application, with suitable dosages of simple compounds .

The question is raised: which mechanism of the compounds used for our tests is instrumental in triggering the symptoms observed in the ovary? Since the detected changes fully coincide with those resulting during the pregnancy reaction of ZONDEK-ASCHHEIM and FRIEDMANN respectively,and with the symptoms respectively which usually occur after the administration of the gonadotropic hormones of the hypophysis, it is highly probable that the compounds used by us cause the changes observed in the ovary likewise through the front lobe of the hypohysis. However, since the applied compounds include organic and inorganic compounds as well as ammoniacal and non-ammoniacal compounds, it is obvious that their specific chemical composition cannot be held responsible for the effect on the hypophysis, or for the effect on the ovary respectively, through the hypophysis. It is a shared characteristic of these compounds that they shift the acid base equilibrium of the organism towards <u>acidity</u>. This has been confirmed by our earlier investigations and by the examinations performed by others.

We are therefore of opinion that the minor periodical shift of the acid base equilibrium towards acidity ,resulting from the effect of the applied compounds, most probably stimulates the corresponding cells of the front hypophysis lobe, which then react with intensified gonadotropic hormone production; the latter cause follicle maturation, the formation of coropra lutea and ovary enlargement. This coincides with the findings of SELYE and HERLANT during "alarm reaction", when after the intravenous injection of hydrochloric acid an increased number of basophilic cells of the front hypophysis lobe was observed, together with follicle maturation in the ovary.

We mentioned earlier that follicle rupture occurs in tame rabbits only 10 to 18 hours after mating. This is due to the fact that in rabbits (and in cats) the front hypophysis lobe produces follicle-maturing hormone only under the effect of the nervous excitation caused by the mating. HARRIS (1941) injected cuprum-acetate into the third cerebral chamber of tame rabbits; this triggered ovulation through the excitation of the hypothalamicus centers. It is necessary in this context to take the fact into consideration as well that the compounds administered by us, by shifting the acid base equilibrium towards acidity, excite the hypothalamicus center of the hypophysis, which causes the front hypophysis lobe to produce gonadotropic hormones; the latter then cause the symptoms observed in the ovary.

It can be assumed on the basis of our rabbit tests that the ovaries of other animals and eventually those of women the function of which is reduced can likewise be induced to regular or intensified functioning with the suitable therapeutic administration of the used compounds or of other compounds having an acidotic effect. However, further tests are needed to reach a final decision in this respect.

Our findings concerning geese indicate that the ovulation process of poultry can be accelerated with the applied treatment, eventually making increased egg production possible. The confirmation of the above would be of noteworthy economic importance.

I wish to state, for the sake of completeness, that we were able to verify, besides changes of the ovary, hyperthrophy of the suprarenal glands, a gradual enlargement of the teats (proliferatic), followed first by plasma secretion and later by milk secretion which lasted for months, enlargement of the womb, enlargement of the hypophysis,etc. I will report in detail on these symptoms in another paper.

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Fazekas- '49
Bib No. 111

Orvosi Hetilap 90:777- 781. 1949.

A szegedi Tudományegyetem Törvényszéki Orvostani Intézetének közleménye.
Intézetvezető: Fazekas I. Gyula dr. egyet. magántanár.

Kísérleti adatok a petefészekműködés befolyásolására egyszerű vegyületekkel*

Irta: FAZEKAS I. GYULA dr.

A csökkent működésű petefészek működésének pótlására vagy helyreállítására az utóbbi két évtizedben főként a folliculus hormont és a corpus luteum hormont vagy a hypophysis mellső lebenyének gonadotrop hormonjait (prolan A, prolan B) alkalmazzák, több-kevesebb sikerrel. Némelyek a stílben származékok adagolásától észleltek jó eredményt. Egyes szerzők a petefészkek, mások a hypophysis Röntgen besugárzásától, némelyek pedig szénhydratmentes étrendben (húsok) dietatol (Loralek) stb. láttak kedvező eredményt ezen a téren. Mivel ezen eljárások alkalmazásával egyrészt nem mindig lehet a petefészek működését helyreállítani, másrészt pedig különösen a hormon kezelés meglehetősen költséges, kívánatos olyan kezelési mód után kutatni, amely az eddigi eljárások hátrányait kiküszöbölné.

Korábbi munkáimban ismertettem azokat a vizsgálataimat, amelyekben kimutattam, hogy bizonyos vegyületek megfelelő adagolására a különböző állatok mellékveséi megnagyobbednak és azok kéregállománya fokozottabban működik a rendesnél. Ezen kísérleteim során olyan megfigyeléseket tehettem, amelyek arra utaltak, hogy minden hormonadás nélkül, egyszerű vegyületek megfelelő adagolása által is lehetséges a petefészkek működését befolyásolni.

Első ilyenirányú megfigyeléseimet NH_4OH-

* Az Orvos-Egészségügyi Szakszervezet szegedi Tud. Csoportjának 1949. év február 2-iki ülésén tartott előadás.

val kezelt 96 házinyúlon tettem. E kísérleteinkben 2500—3500 gr súlyú, elkülönítve tartott, nemileg érett (8—14 hónapos) nőstény házinyulaknak másodnaponkint per os 50—60 ccm ½%-os NH_4OH-t adva, 1—3 hónap multa levágtuk, amikor mellékvesekéreg-túltengés mellett, a petefészkeken folliculusérést, vérzéses folliculusokat és sárgatest-képződést észleltünk, bizonyos fokú megnagyobbodás kíséretében. Második megfigyeléseink libákon történtek, amikor 10 egyköltésből származó libának mellékvesemüködés-fokozás céljából másodnaponkint 50—70 ccm ½%-os NH_4—OH-t adagoltunk gyomorsondán keresztül, fokozatosan emelkedő adagokban 4 héten át, más 10, ugyanazon költésből származó testvérlibát pedig kezelés nélkül kontrollként ugyanúgy táplältunk. A libákat 4 hét multa leölve, az NH_4OH-val kezelt libák petefészkében számos diónyi és ennél nagyobb tojássárgáját (petet) találtunk, amelyek egy-egy libában együttesen jókora férfiökölnyi tömeget képeztek. Ezzel szemben a kezelés nélkül ugyanúgy táplált libák petefészkében alig zöldborsó nagyságú peték (tojássárgája) voltak.

Ezen előzetes megfigyelések alapján újabb kísérleteket végeztünk, amelyekben nemileg érett (8—14 hónapos), nőstény, virgo intacta, elkülönítve tartott azonos fajú (csincsilla) házinyulakat alkalmaztunk és azonos táplálékon tartottunk. Házinyulak kiválóan alkalmasak olyan kísérletekre, amelyekben a petefészkek működését mesterséges úton kívánjuk befolyásolni. *Van Beneden* és *Heape*, valamint mások vizsgálataiból ugyanis tudjuk, hogy házinyulakon az ovulatio, illetve folliculus repedés csak a párzás után jön létre.

Ismeretes azonban, hogy házinyulakon »steril hágás« után is bekövetkezik a folliculusrepedés, aminek nyomán corpora luteak képződnek. Az ilyenkor keletkező corpus luteumok hatására a genitaliákon jellemző elváltozások (méhnagyobbodás, decidua képződés, emlő-megnagyobbodás) jelentkeznek. Ezt az állapotot »álterhességnek« vagy »látszatterhességnek« szokás nevezni. Az álterhességi állapot *Ancel* és *Bouin* szerint 13—14 napig, *Hammond* szerint 16—19 napig, *Knaus* szerint pedig 16 napig tart, amikor a sárgatestek degeneratiója miatt visszafejlődik. Ezért az »álterhesség« elkerülése végett a petefészek működésére, illetve a genitaliák állapotára vonatkozó kísérletek előtt a házinyulakat legalább 1 hónapig elkülönítve kell tartani.

Mi az alábbi kísérletek során úgy a kezelésre szánt, mint a kontroll-nyulakat már 5 hónapos korukban, tehát az ivarérés (6 hónap) előtt 1 hónappal elkülönítettük és a kísérletbefogás előtt is már 3—8 hónapig elkülönítve tartottuk. Az elkülönítés természetesen a kísérlet ideje alatt is tartott. Ezzel az eljárással azt kívántuk elérni, hogy különböző korú olyan petefészkeket vizsgálhassunk, amelyek ivarérettek ugyan, de bennük természetes okokból tüszőérés és repedés, valamint corpus luteumképződés még ne legyen, és így a kezelés hatását a legexactabb módon tudjuk lemérni.

Az első kísérletsorozatban 2500—3500 gr kezdeti súlyú 8—14 hónapos nőstény házinyulakat 5—7 állatból álló (de külön tartott) csoportokba osztottuk s minden csoport állatait más-más vegyülettel kezeltük. Minden állat fokozatosan emelkedő adagokban másodnaponkint testsúlykilogrammenkint 0.1—0.2 gr hatóanyagot kapott 100—150 ccm ivóvízben oldva. A kezelés 5 hónapon át tartott, úgy, hogy három heti kezelés után mindig egy heti kezelésmentes időszakot iktattunk közbe. Kezelésre a következő vegyületeket alkalmaztuk: Ammoniumchlorid, ammoniumsulfát, ammoniumcarbonat, natrium-ammoniumphosphat, ammoniumacetat, ammoniumlactat, calciumchlorid, acidum hydrochloricum (25%-os), acidum lacticum cc., acidum aceticum, natrium dihydrophosphat, ammonium hydrophosphat. Az alkalmazott kezeléssel az állatok savbasis-egyensúlya időszakosan enyhébb mértékben a savi irányba tolódik el, a köznapi értelembe vett (súlyosabb) acidosis azonban nem jön létre. Az állatok mindvégig jó étvággyal táplálkoztak és hízták. Kontrollként 50 hasonló korú, fajú és kezdeti súlyú virgo intacta nőstény házinyulat ugyanolyan minőségű és mennyiségű táplálékon ugyanúgy elkülönítve tartottunk.

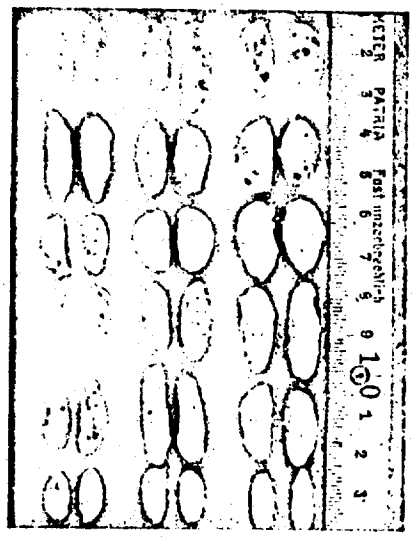
A kezelt és kontroll-nyulakat 5 hónap multa légemboliával megöltük és azt találtuk, hogy a

Házinyulak petefészkének megnagyobbodása különböző vegyületekkel történt kezelés következtében

Sorszám	A kezelés módja	Az esetek száma	A 2 ovarium súlya cg			A 2 ovarium súlygyarapodása középértékben		S. d.
			minimum	maximum	középérték	cg	%	
1.	Kontroll	50	15	52	38.60	–	–	
1.	Ammonium hydroxyd	96	42	150	93.24	24.58	63.57	6.02
2.	Ammonium chlorid	50	53	108	70.82	32.16	88.36	5.82
3.	Ammoniumsulfát	5	68	128	95.50	56.84	147.00	7.03
4.	Ammonium carbonát(!)	7	60	92	80.70	52.04	134.60	6.31
5.	Na, ammon. phosph.	7	67	111	95.66	57.00	150.00	9.20
6.	Ammonium acetat	6	54	121	87.30	48.64	125.81	8.32
7.	Ammonium lactat	5	68	146	104.00	66.00	173.30	9.24
8.	Calcium chlorid	5	60	146	102.20	63.54	164.35	8.00
9.	Ac. hydrochloricum	5	73	115	93.60	54.94	142.10	7.38
10.	Ac. lacticum	5	64	118	96.40	57.74	150.00	7.05
11.	Ac. aceticum	6	57	102	87.02	48.96	125.80	7.14
12.	Natrium dihydrophosphat	5	92	162	126.33	87.67	220.35	11.32
13.	Ammon. hydrophosphat	5	81	132	112.00	73.34	190.47	8.27

zelt állatok petefészkei a kontrollokéhoz viszonyítva minden esetben kisebb-nagyobb mértékben megnagyobbodtak, súlyosabbak voltak, felületükön pedig már szabadszemmel számos megnagyobbodott érett és bevérzett folliculus, vagy corpus luteum volt látható.

A táblázat adatai meggyőzően mutatják, hogy az alkalmazott vegyületekkel végzett kezelés hatására a petefészkek jelentékeny, középértékben 63.57—229.35%-os súlynövekedése következett be. A súlynövekedés significans voltát (S. D.) elvégzett statisztikai (valószínűségi) számítások igazolják. A térfogatos növekedést, valamint az érett folliculusokat, bevérzett folliculusokat és a corpus luteumokat jól szemlélteti a mellékelt (1.



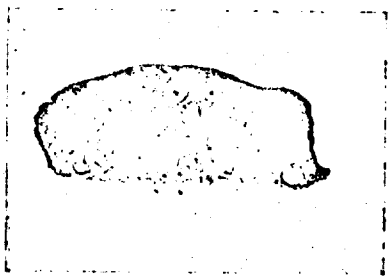
1. kép. Megnagyobbodás, folliculus érés és bevérzés, corpus luteum képződés különböző vegyületekkel 5 hónapig kezelt virgo intacta házinyulak petefészkein.

I. sorban	Calciumchlorid kezelés	93 cg	127 cg	146 cg	
II. »	Ammonium acetat »	83 »	100 »	121 »	
III. »	» lactat »	68 »	100 »	146 »	
IV. »	» sulfát »	85 »	88 »	128 »	
V. »	natrium-ammonium-phosphat kezelés	80 »	90 »	111 »	
VI. »	kezelés nélkül (kontr.)	37 »	40 »	40 »	

az) fénykép, amelyen a legalsó (VI.-ik) sorban az azonos korú és súlyú kezeletlen kontroll-nyulak petefészkei, az I—V-ik sorban pedig sorrenkint más-más vegyülettel kezelt nyulak megnagyobbodott petefészkei láthatók.

Szövettani vizsgálat. A nem kezelt, *kontroll* házinyulak petefészkének metszetein nagyszámú, sűrűn egymás mellett elhelyezkedő primaer folliculus közt számos (egy-egy metszetben 10—14) érőfélben levő, de még nem érett, aránylag nem nagy folliculus látható, úgy, hogy az interstitialis petefészekmirigyek (velőállomány) aránylag csak igen kis területre szorítkoznak. Teljesen érett, repedésre kész folliculusok nem észlelhetők. (2. kép.)

Ezzel szemben az 5 hónapon át *kezelt*, elkülönítve tartott virgo intacta házinyulak megnagyobbodott petefészkének nagyobb részét az interstitialis petefészekmirigyek (velőállomány) alkotják, amelyeknek sejtjei szembetűnően nagyobbak a normálisnál és számuk is lényegesen megszaporodott, protoplasmájukban a lipoidok megszaporodtak, kisebb-nagyobb cseppeket képeznek, a sejtmag pedig gyakran oldalra tolódott. Némely esetben egy-egy foltszerű területen ezek a sejtek annyira lipoid dúsak, hogy erősen hasonlítanak a lutein sejtekhez. Feltűnő ennélfogva, hogy az állatok egy részének petefészkében számos érett folliculus, vérbőség és *bevérzett folliculus* (3. kép), más állatok petefészkeiben pedig számos *corpus luteum* (4. kép) látható. Érett és bevérzett tüszők, valamint corpora luteak nemcsak a felszínen, hanem a mélyebb rétegekben is vannak. Egyes petefészekben a kéregállomány megkeskenyedett, másokban nem. A kezelt nyulak petefészkeiben folliculus degeneratiót vagy petesejt degeneratiót nem észleltünk. Ez



2. kép. 3000 gr súlyú virgo intacta 13 hónapos nem kezelt (kontroll) házinyúl petefészkének metszete. A két petefészek együttes súlya 25 cg.

3. kép. Bevérzett és érett folliculusok 13 hónapos virgo intacta házinyúl petefészkében *5 hónapig tartó calciumchlorid kezelés után.* A két petefészek együttes súlya 146 cg. Kezdeti testsúly: 3100 gr. Testsúly a kezelés végén: 4200 gr. Haematoxylin-eosin festés.

4. kép. Corpus luteumok 13 hónapos virgo intacta házinyúl petefészkében, *5 hónapig tartó ammonium lactum kezelés után.* A két petefészek együttes súlya: 146 cg. Kezdeti testsúly: 3100 gr. Testsúly a kezelés végén: 4700 gr. Van Gieson festés.

amellett látszik szólani, hogy az alkalmazott kezelés nem károsítja a folliculusokat, illetve a petesejteket. A kezelt nyulak petefészkein szembetűnő, hogy a primaer folliculusok és az érőfélben levő folliculusok a kontroll petefészkekhez viszonyítva ritkábban, egymástól távolabb helyezkednek el. Ez azzal magyarázható, hogy egyrészt a petefészek tömegének és felületének megnagyobbodása miatt a folliculusok egymástól mintegy szétterülnek és nagyobb területen helyezkednek el, másrészt pedig a kezelés során bekövetkező fokozott folliculus érés miatt a folliculusok absolut száma is természetszerűleg csökken.

Az elmondottakból megállapítható, hogy az észlelt petefészekmegnagyobbodás egyrészt az interstitialis petefészekmirigyek túltengésére és azok lipoid tartalmának szaporodására, másrészt pedig a folliculusok megnagyobbodására, érésére, bevérzésére, illetve a corpora luteák képződésére vezethető vissza.

Másik kikérletsorozatban az előbbiekkel azonos kísérleti körülmények mellett ugyancsak 5–7 (8–10 hónapos) nyulat ugyanazon vegyületekkel (80–100 adag) kezeltünk és 14–16 hónapig figyeltünk meg, majd légembóliával történt leölésük után megvizsgáltuk petefészkeiket. Ezen állatok petefészkein lényegileg hasonló jelenségeket láttunk, mint az 5 hónapig tartó kísérleti idő után, miért is ezek részletes ismertetését mellőzöm. Csupán annyit jegyzek meg, hogy degeneratiós jelenségeket ezen állatok petefészkein sem észleltünk.

Az irodalmi adatok szerint a petefészken folliculus érlelő és sárgatestképző hatást elsősorban a hypophysis mellső lebenye által termelt gonadotrop hormonok hatására észleltek.

Nagyszámú vizsgálatok kétségtelenül bebizonyították, hogy a petefészkek működését a hypophysis mellső lebenye hormonalis úton irányítja.

A szerzők egy része különböző kísérletek alapján azt tartja, hogy a hypophysis basophil sejtjei termelik a folliculus érlelő hormont, az acidophil sejtek pedig a corpus luteum képző hormont. *Herlant* a *Selye*-féle alarm-reactióban megszaporodott basophil sejtek átültetése alkalmával folliculus érlelő hatást észlelt. *Giroud* és *Martinet* ökör- és sertés-hypophysis basophil sejteket tartalmazó zónájának nőstény ivarérett patkányokba történt implantálására a petefészek jelentékeny megnagyobbodását, folliculus-érést és corpus luteum-képződést figyelt meg. Az *acidophil* sejtcsoport átültetésére csak kisfokú petefészek megnagyobbodást és csekély folliculus-megnagyobbodást láttak kifejlődni, corpus luteumok azonban nem keletkeztek. Kísérleteik alapján a hypophysis mellső lebeny basophil sejtjeinek tulajdonítanak folliculus-érlelő hatást, a corpus luteum-képződésben pedig ugyancsak szerepet tulajdonítanak a basophil sejteknek is.

Hohlweg fiatal patkányok, *Klaften*, *Pataky* és mások ivarérett, elkülönített házinyulak petefészkén nagyobb adag *folliculus hormon* befecskendezésre corpus luteum-képződést figyeltek meg. Ezt úgy magyarázzák, hogy a tüszőhormon nagy mennyiségben gátolja a hypophysis mellső lebenyének tüszőérlelő hormon (prolan A) termelését, ezzel szemben elsőségíti a sárgatestképző hormon (prolan B) képződést. *Baumann* terhes nő felforralt, tehát prolant nem tartalmazó vizeletének befecskendezésre állatok petefészkében sárgatestképződést észlelt, amit a terhes nő vizeletében levő nagymennyiségű folliculinnak a hypophysisen keresztül kifejtett hatására vezet vissza, úgy, mint *Hohlweg* és mások. *Zondek*, *Clauberg* és mások vizsgálatai szerint a sárgatestképződést mindig bizonyos fokú tüszőérés előzi meg, amit a prolan A vált ki. *Axel–Westmann* vizsgálatai is rámutattak arra, hogy tüszőérés nélkül nincs sárgatestképződés.

Julesz ketogen diétán tartott nők és férfiak vizeletének infantilis fehér egerekbe oltása után az állatokban folliculus-érést és vizenyős méhduzzadást észlelt, folliculus bevérzés és corpus luteum képződés azonban nem jött létre. *Julesz* e jelenséget úgy magyarázza, hogy a ketosis a hypophysis mellső lebenyét fokozott prolan A termelésre ingerli.

Megemlítjük még, hogy *Luigi* infantilis házinyulakon a hypophysis, *Freedgood* és *Pincus* pedig tengeri malacokon a nyaki sympaticus elektromos ingerlése által idézett elő jelentős tüszőérést.

Saját ismertetett vizsgálataink eredményei azt mutatják, hogy hormonadás nélkül, egyszerű vegyületek megfelelő adagolásával is lehetséges házinyulak petefészkén folliculus-érést, folliculus-bevérzést és corpus luteum-képződést létrehozni.

Felvetődik a kérdés, hogy a kísérleteinkben alkalmazott vegyületek milyen mechanismus révén váltják ki a petefészken észlelt jelenségeket. Mivel a talált elváltozások mindenben megegyeznek, a *Zondek-Aschheim*, illetve a *Friedmann*-féle terhességi reactióban keletkezőkkel, illetve azokkal a jelenségekkel, amelyek a hypophysis gonadotrop hormonjainak adagolására szoktak létrejönni, minden valószínűség amellett szól, hogy az általunk alkalmazott vegyületek is a hypophysis mellső lebenyén keresztül idézik elő a petefészken észlelt elváltozásokat. Mivel azonban az alkalmazott vegyületek közt szerves és szervetlen, továbbá ammoniák és nem ammoniak vegyületek szerepelnek, nyilvánvaló, hogy nem specialis vegyi összetételük tehető felelőssé a hypophysisre, illetve azon át a petefészekre gyakorolt hatásért. E vegyületek közös tulajdonsága azonban az, hogy megfelelő adagokban a szervezet savbasis egyensúlyát *sari irányba* tolják el. Ezt saját korábbi és mások vizsgálatai bizonyítják. Ezért nézetünk szerint a legnagyobb valószínűséggel a savbasis-egyensúlynak az adagolt vegyületek hatására bekövetkező mérsékelt fokú időszakos savanyú irányú eltolódása izgatólag hat a hypophysis mellső lebenyének megfelelő sejtjeire, amelyek azután fokozottabb gonadotrop-hormontermeléssel válaszolnak és ez utóbbiak idézik elő a folliculus-érést, sárgatestképződést és petefészekmegnagyobbodást. Összhangban áll ez *Selye* és *Herlant* alarm reactióban tett észleletével, amikor intravenás sósavbefecskendezés után a hypophysis mellső lebeny basophil sejtjeinek megszaporodása mellett mellékvesetúltengést és a petefészken folliculus-érést figyeltek meg.

Említettük már, hogy házinyulakban folliculus-repedés csak a párzás után 10–18 óra múlva következik be. Ennek oka az, hogy nyulakban (és

[illegible]ackákban) a hypophysis mellső lebeny tüszőérlelő hormont csak a párzás okozta idegizgalom hatására termel. *Harris* (1941) házinyulak harmadik agykamrájába cuprum-acetat oldatot fecskendezett be, ami a hypothalamicus központok ingalma révén ovulatiót váltott ki. Ennek alapján arra is lehet gondolni, hogy az általunk adagolt vegyületek a savbasis-egyensúly savi irányba tolása által izgatják a hypophysis hypothalamicus központját és ez váltja ki a hypophysis mellső lebenyének gonadotrop hormontermelését, ez utóbbi pedig a petefészken észlelt jelenségeket.

Nyúlkísérleteink alapján feltehető, hogy az alkalmazott vagy más acidoticus hatású vegyületek megfelelő gyógyszeres adagolása által egyéb állatok és esetleg nők csökkent működésű petefészke is rendes vagy fokozottabb működésre serkenthető. Ennek eldöntésére azonban még további vizsgálatok szükségesek.

Libákon tett megfigyeléseink arra mutatnak, hogy az alkalmazott kezeléssel szárnyasok petefészki folyamatát gyorsítani lehet, ami esetleg a tojástermelés fokozását tehetné lehetővé. Ennek igazolása figyelemreméltó gazdasági jelentőséggel bírna.

A teljesség kedvéért megemlítem, hogy kezelt nyulainkon a petefészek elváltozásai mellett a mellékvesék túltengését, az emlők fokozatos megnagyobbodását (proliferatio), majd előbb savó, később pedig hónapokon át tartó tejelválasztást (secretio), méhmegnagyobbodást, a hypophysis megnagyobbodását stb. állapíthattuk meg. E jelenségekről részletesebben más munkánkban számolunk be.

Összefoglalás: 1. Virgo intacta, elkülönítve tartott nemileg érett házinyulaknak másodnaponkint 3—5—16 hónapon át 13 olyan szerves és szervetlen ammoniak és más vegyületeket adagoltunk, amelyek közös tulajdonsága az, hogy a szervezet savbasis-egyensúlyát a savi irányba tolják.

2. E kezelés hatására a házinyulak petefészkén 63—229%-os súlynagyobbodás, térfogatnövekedés, folliculus-érés, folliculus-bevérzés és corpus luteum-képződés következett be.

3. A petefészek térfogatának növekedése részben az interstitialis petefészekmirigyek (velőállomány) hyperplasiájára, részben pedig a folliculusok megnagyobbodására és a corpus luteumok képződésére vezethető vissza.

4. Az interstitialis petefészekmirigyek sejtjeinek megnagyobbodása azok lipoid tartalmának jelentékeny megszaporodására vezethető vissza. Emellett e sejtek száma is megnövekedett.

5. Libákon tett megfigyeléseink szerint az alkalmazott kezeléssel az állatok petefészkén feltűnő petefészgyorsulás és fokozódás volt észlelhető, ami arra utal, hogy ily módon esetleg szárnyasok tojástermelését fokozni lehetne, ami gazdasági szempontból bírna jelentőséggel. Erre nézve azonban még további vizsgálatok szükségesek.

6. Vizsgálataink eredménye és az irodalmi adatok alapján arra lehet következtetni, hogy az észlelt petefészekelváltozások minden valószínűség szerint a hypophysis mellső lebenyének a kezelés hatására bekövetkező fokozott gonadotrop-hormon termelésének következményei.

7. Nézetünk szerint az alkalmazott vegyületek nem specifikus vegyi tulajdonságaik révén hozzák létre a hypophysis mellső lebenyének fokozott gonadotrop-hormon termelését, hanem azáltal, hogy a szervezet savbasis-egyensúlyát időszakonkint és mérsékelt fokban a savanyú irányba tolják el, ami azután vagy közvetlenül hat a hypophysisre, vagy pedig a hypophysis hypothalamusban levő idegközpontjának izgatása révén váltja ki a fokozott gonadotrop-hormon termelést.

8. Kísérleteink eredményei alapján feltehető, hogy az alkalmazott, vagy más acidotikus hatású vegyületek megfelelő adagolása által más állatok és esetleg nők csökkent működésű petefészkei is fokozottabb működésre serkenthetők és ezáltal azok tevékenysége rendessé tehető. Ennek eldöntésére még további vizsgálatok szükségesek.

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Endokrinologie 32:45-57. 1954.

Fazekas-'54
Bib No. 113

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THE EFFECT OF THE PARATHYROID GLAND FUNCTION (Serum % Ca and % P) BY ACIDOTIC COMPOUNDS

by I. Gy. Fazekas

Mac Callum and Voegtlin detected a reduction in the blood-Ca content in dogs after removal of the parathyroid glands. Greenwald as well as Hastings and Murray, Reed, Zachy and Payte, on the other hand, observed that the P content of the blood in such animals increased. Collip et al. found that after injection of parathyroid extract (pharahormone) there was an increase in the Ca content of the blood serum both in normal dogs and cats and in parathyroidectomized dogs and cats. It is known that in humans suffering from Recklinghausen disease (osteitis fibrosa generalisata) caused by an enlargement and increased function of the parathyroid glands the Ca content of the blood serum increases considerably, the inorganic P content decreases considerably, however. Similar changes in the serum Ca and P content were observed also in acute hyperparathyroidism (Olivier, Smith , Hanes). In various cases of hypoparathyroidism there is an increase in the serum p. also in humans along with a decrease in the Ca content of the blood serum. The parathyroid glands therefore play a first-class role in the regulation of the Ca and P metabolism in the organism.

Fazekas determined that the parathyroid glands of rabbits enlarged by 171 to 294% through lengthy application of acidotic-acting compounds. The histological finding indicates the increased function of the parathyroid glands which have been thus enlarged . This cannot be decided, however, solely based on the histological picture. In order to investigate the function of the parathyroid glands thus enlarged new animal experiments were implemented. Since according to the medical data published there is a close relationship between the Ca and inorganic P content of the blood and the parathyroid function, it was to be assumed that information could be obtained,by systematic investigation of the blood Ca and P content in animals which had been treated with compounds having an acidotic effect and causing a parathyroid hypertrophy, concerning the function of the parathyroid glands.

In our tests 72 similar-type (Chinchilla) and similar-aged (8 to 10 month old) female and male rabbits with an initial weight between 2500 to 3000 grams were treated for from 5 to 26 months with compounds producing an acidotic effect. Fifty animals, however, which did not receive such treatment, were used as control animals. The animals were fed with the same amount of oats and dry clover. The following compounds were administered: ammonium chloride, ammonium sulphate, ammonium hydrophosphate, ammonium acetate, ammonium lactate, sodium hydrophosphate, acidum hydrochloricum (hydrochloric acid), acidum lacticum (lactic acid), acidum aceticum (acetic acid),and calcium chloride. Six animals were treated with each compound. Every animal was given twice a day for a period of three weeks in weekly increasing doses 0.1-0.2 g per kilogram of body weight of the corresponding compound dissolved in 50 to 80 ccm of drinking water. After that there follows a two week period during which no treatment was given. Thus the treatment period and treatment pause were repeated for 5 to 26 months. Six rabbits were treated in the same way with the following compound mixture: 30 g ammonkum chloride, 5 g ammonium acetate, 5 g ammoniumhydrophosphate, 10 g calcium chloride, 5 g hydrochloric acid, 5 g lactic acid, with 1000 g of drinking water.(aquae fontis) . . In 1 ccm of this solution there is 0.06 g of effective substance mixture. These animals were treated for 16 . Six other rabbits were treated with the same compound mixture and in the same way but for 26 months.

In the laboratory animals the Ca content of the blood serum and the inorganic P content of the total blood were determined before, during and after the treatment for a period of two weeks. The control rabbits were also subjected to the same tests. The Ca (calcium) content was determined in 1 ccm of blood serum by means of the oxydimetric method of Clark and Collip. The inorganic P (phosphorus) content of the blood, however, was determined in 0.2 ccm full blood with the colorimetric method of Kuttner and Cohen by means of a Stankosch electrophotometer. The mean value of 2 determination processes conducted in parallel always gave the result. For purposes of brevity the individual data will not be reported, but in the attached table only the mean values of the individual animal groups will be given. Since in the 11th animal group during the first 12 months the same characteristic serum Ca and P changes were found as in the 1st to 10th animal groups, these data will not be discussed individually, but only the results of the 11th, 12th and 13th treatment periods (13-16 months). In the case of the 12th animal group which was treated with the compound mixture the blood serum Ca and inorganic P content of the blood over the course of the 18-month treatment showed changes of the same nature as in the preceding groups. In the case of the 12th animal group a pause of 3 months was given after 18 months of treatment. During this time the serum Ca and inorganic P returned to the normal initial values. Then the treatment of the animals was continued in the same way for a further 5 months (4 treatment periods).

The detailed evaluation of the various Ca and P changes will be omitted here. As an example we would like to cite only the evaluation of the treatment with ammonium sulphate as well as the 26-month treatment with the compound mix.

Ammonium Sulphate Treatment

1. Treatment period. Serum-Ca. Prior to the treatment 14.92 mg%. After the first week of treatment 9.39 mg %, which signifies in comparison to the normal initial value a drop of 5.53 mg % (37.06%). After two weeks of treatment 17.35 mg % , that is 2.45 mg % more (16.4%), after three weeks of treatment 18.45 mg %, i.e. 3.53 mg % (23.6) more. After a one-week treatment pause 19.88 mg % , after a two week pause 16.42 mg % , which in comparison with the normal initial value signifies an increase of 4.69 mg % (31.9%) and 1.52 mg % (10.0%). Blood-P: Prior to the treatment 3.31 mg %. After one week of treatment 4.13 mg %, which means an increase of 0.82 mg % (26.1%). After two weeks of treatment 2.19 mg % , after 3 weeks' treatment 1.99 mg %, that is to say 1.12 mg % (33.8%) and 1.32 mg % (39.9%) less than the normal initial value. After a one-week treatment pause 3.62 mg % , after a two-week pause 4.97 mg % , i.e. 1.63 mg % (81.9%) and 2.98 mg (149.7 %) greater than after 3-week treatment and 0.31 mg % (9.3%) and 1.66 mg % (50.1%) greater than the normal initial value.

II. Treatment period. Serum-Ca: after 1-week treatment 16.24 mg % , after 2-week's treatment 21.44 mg %, after 3 weeks 23.11 mg %, i.e. 2.32 mg % (15.55%), 6.52 mg % (43.6%) and 8.19 mg % (54.9%) more than the normal initial value and 5.02 mg % (30.5%) and 6.69 mg % (40.7) greater than the value after 2-week pause of the preceding treatment period. After one week treatment pause 20.77 mg %, after two-week pause 16.26 mg %, i.e. 5.85 mg % (39.2%) and 1.34 mg % (8.9%) greater than the normal initial value. This signifies in comparison with the initial treatment value an increase of 2.34 mg % (10.1%) and 6.85 mg % (29.9%). Blood-P: after one week treatment 2.35 mg % , after two week treatment 1.44 mg %, after three week treatment 1.62 mg %, which amounts to 0.96 mg % (29.0%), 1.87 mg % (56.5%) and 1.69 mg % (51.0%) less than the normal initial value. After a one-week pause 3.14 mg %, after two-week pause 3.2 mg % , i.e. 0.17 mg % (5,1 %) and 0.11 mg % (3.3%) less than the normal initial value.

Third Treatment Period. Serum-Ca: after one-week treatment 16.9 mg % , after two weeks 20.51 mg %, after three weeks treatment 22.66 mg %. These values are 1.98 mg % (13.3%), 5.69 mg % (37.6%) and 7.77 mg % (51.8%) greater than the normal initial value. After one-week treatment pause 20.1 mg %, after two-week pause 17.23 mg %, which is 5.18 mg % (35.3%) and 2.21 mg % (14.8%) greater than the normal initial value, but on the other hand 2.56 mg % (11.2%) and 5.43 mg % (23.9%) less than the value after three weeks of treatment of this period. Blood-P: after one-week treatment 2.59 mg %, after

two-week treatment 2.22 mg % , after three-week treatment 1.8 mg %. These values are 0.62 mg % (15.7%), 1.09 mg % (32.9%) and 1.51 mg % (45.6%) lower than the normal initial value. After one-week treatment pause 2.66 mg %, after 2-week pause 3.46 mg %, i.e. 0.65 mg % (16.6%) less and 0.15 mg % (4.5) more than the normal initial value. The P value therefore returned somewhat to the normal value.

Fourth Treatment Period. Serum-Ca: After one-week treatment 18.31 mg % , after two-week treatment 20.42 mg %, after 3-week treatment 22.14 mg %, i.e. 3.39 mg % (22.7%), 5.50 mg % (36.8%) and 7.22 mg % (48.2 %) higher than the normal initial value and 1.08 mg % (6.2%), 3.19 mg % (18.5%) and 4.91 mg % (28.5%) greater than the value after the two-week treatment pause of the preceding treatment period. After one-week treatment pause 18.60 mg % , i.e. 3.68 mg % (24.6%) greater than the normal initial value, but 3.54 mg % (15.9%) less than after the three week treatment of this period. Blood-P: After 1-week treatment 2.23 mg %, after two weeks of treatment 2.32 mg %, after three weeks of treatment 1.75 mg %, i.e. 0.98 mg % (29.6%), 0.99 mg % (29.9%) and 1.56 mg % (47.1%) less than the normal initial value. After one-week treatment pause 2.63 mg % , i.e. 0.82 mg % (45.2%) more than the value after three-week treatment of this period, but 0.68 mg % (20.5%) less than the normal initial value.

Twelfth Treatment Group (compound mixture)

Eighteenth (XVIII) treatment period. Serum-Ca: Prior to the treatment 15.81 mg %, after one week treatment 11.92 mg %, which signifies in comparison to the normal initial value a decrease of 3.89 mg (24.6%) . This decrease was not as great, however, as at the start of the first treatment (37.2%). After two-week treatment 17.81 mg %, after three-week treatment 22.86 mg %, i.e. 1.0 mg % (6.3%) and 7.05 mg % (44.59%) more than the normal initial value. After one week treatment pause 20.27 mg %, after two-week pause 16.35 mg %, i.e. 2.59 mg % (11.3%) and 6.51 mg % (29.3%) less than after three-week treatment of this period, but 4.46 mg % (28.2%) and 0.54 mg % (3.4%) more than the normal initial value. Blood P: before the treatment 3.72 mg %. After one week of treatment 4.58 mg %, i.e. 0.86 mg % (23.1%) more than the normal initial value. After the second week of treatment 2.77 mg %, after the third week of treatment 1.88 mg % , i.e. 0.95 mg % (25.5) and 1.84 mg % (49.4%) less than the,normal initial value. After one week treatment pause 3.07 mg %, i.e. 0.85 mg % (22.8%) and 0.65 mg % (17.4%) less than the normal initial value, but 0.99 mg % (52.6%) and 1.19 mg % (63.1%) more than after the third week of treatment of this period.

Nineteenth (XIX) Treatment Period. Serum-Ca: After one week treatment 15.66 mg %, i.e. almost the same as the normal initial value. This is evidence of the fact that the organism was already able to ward off the Ca-reducing effect of the treatment. After two week treatment 19.84 mg %, after 3 week treatment 22.63 mg %, which in comparison to the normal initial value signifies a Ca increase of 4.03 mg % (23.4%) and 6.82 mg % (43.1%). After one week treatment pause 20.60 mg % and after 2 week pause 16.41 mg %, which in comparison with the value after the 3 weeks of treatment of this period signifies a drop of 2.03 mg % (8.4%) and 6.22 mg % (27.4%), but which amounts to 4.79 mg % (30.3%) and 60 mg % (3.7%) more than the normal initial value. Blood-P: After one-week treatment 3.23 mg %, after 3-week treatment 2.30 mg %, i.e. 0.49 mg % (13.1%) , 1.28 mg % (34.4%) and 1.-2 mg % (38.1%) less than the normal initial value. After a one week pause 3.05 mg % and after a two week treatment pause 3.39 mg %, i.e. 0.67 mg % (18.0%) and 0.33 mg % (8.8%) less than the normal initial value, but o.75 mg % (32.6¢) and 1.09 mg % (47.3%) more than after the third week of treatment of this period.

Twentieth (XX) Treatment Period. Serum-Ca: After one week treatment 16.93 mg %, after two week treatment 19.88 mg %, after 3 weeks treatment 21.99 mg %, i.e. 1.12 mg % (7.0%), 4.07 mg % (26.3%) and 6.18 mg % (39.0%) greater than the normal initial value. After one week treatment pause 20.06 mg %, after 2 week pause 17.21 mg %, i.e. 1.93 mg % (8.7%) and 4.78 mg % (21.4%) less than after the three week treatment of this period, but 4.25 mg % (26.8%) and 1.40 mg % (9.4%) more than the normal initial value. Blood P: after one week treatment 2.61 mg %, after two week treatment 2.28 mg %, after 3 week treatment 2.10 mg % , signifying 1.11 mg % (29.8%0, 1.54 mg % (41.3%) and 1.62 mg % (43.5%) less than the normal initial value. After one week treatment pause 2.53 mg %, after two week pause 2.75 mg %, i.e. 1.19 mg % (31.9%) and 0.97 mg % (26.0%) less than the normal initial value, but 0.43 mg % (20.4%) and 0.65 mg % (30.9%) more than after the three weeks of treatment of this treatment period.

TWENTY FIRST (XXI) Treatment Period. Serum-Ca: after one week treatment 17.22 mg %, the same value as after two week treatment pause of the previous period, which also means that the organism was able to compensate for the Ca-reducing action of the 1 week treatment. After 2 weeks of treatment 19.38 mg %, after three weeks of treatment 22.05 mg %, which is 3.57 mg % (28.9%) add 6.24 mg % (39.4%) more than the normal initial value. After one week treatment pause 18.94 mg %, i.e. 3.11 mg % (19.7%) less than after the third week treatment of this period, but 3.13 mg % (19.8%) more than the normal initial value. Blood-P: After one week treatment 2.72 mg %, after two week treatment 2.28 mg %, after 3 week treatment 1.78 mg %, i.e. 1.0 mg % (26.5%), 1.44 mg % (38.6%) and 1.94 mg % (52.1%) less than the normal initial value. After one week treatment pause 2.67 mg %, i.e. 1.07 mg % (28.7) less than the normal initial value, but 0.87 mg % (49.4%) more than after three weeks of treatment of this period.

The investigations with the other compounds also showed the same changes in Ca and P. In the case of the control rabbits, however, it was not possible to determine any similar and equivalent changes in the serum Ca and in the inorganic P.

The results show therefore that under the effect of the acidotic compounds which were used , after the first week of treatment of the first period, the serum Ca dropped considerably below the initial value (24% to 37%), but the blood-P increased at the same time 20 to 42% above the normal initial value. This is in agreement with our earlier finding that under the effect of a dose of NH_4OH or NaOH the serum Ca decreases and the blood-P increases, however. In the 2nd week of treatment there is an increase in the Serum-Ca (10-35%) and it reaches its maximum after 3 weeks of treatment (Ca:20-51%; P: 24-50%. After the 1st and 2nd weeks of treatment pause the serum-Ca value gradually decreases in comparison with the value of the 3 week treatment, but usually remains higher even after 2 weeks (7-21%) than the normal initial value, the blood P increases again, however, over the normal (by 50-67%). During the subsequent treatment periods the serum-Ca value never drops below the normal value after the first week of treatment, but rather it remains 12-22% higher during that period of time, the blood P, however, drops considerably (20-30%) below the normal value. In the 2nd and third weeks of treatment thus the increase in the serum-Ca (50-65) and the decrease in the blood P (30-56%) is still more evident than during the same time of the first treatment period. During the course of the two week treatment pause the serum- Ca gradually decreases, but the blood-P increases in comparison with the value of the 3 week treatment. The serum-Ca, however, is then still greater and the inorganic P still lower than the normal initial value, that is to say that the serum-Ca goes higher and the blood P, however, goes lower than before the start of the treatment.

In the case of the rabbits which had been administered the compound mixture for a period 16 months long and in the case of those rabbits which were treated anew after an 18-month treatment and after the subsequent 3-month treatment pause it was possible to observe Ca and P changes of the same nature.

After the laboratory animals were sacrificed we found a 160.8-277.7 % parathyroid gland enlargement (mean values)! In the parathyroid glances , in agreement with our earlier tests, we were able to observe histologically blood filling, increase and enlargement of the bright cells, nucleus division phenomena, i.e. the signs of a hyperfunction.

Other researchers also detected a parathyroid gland enlargement during long-lasting administration of ammonium chloride, glucose and hydroxylamine (acidosis). They did not study the change in the blood Ca and in the blood P, however, and thus they were also not able to prove anything with regard to the functional increase in the expanded (enlarged) parathyroid glands. It is known that in certain cases of chronic kidney insufficiency there occurs a secondary hyperplasia and an increase in the function of the parathyroid glands.

which can then cause characteristic bone changes. The bone system of our laboratory animals, after supplementing with newer tests, was subjected to a histological, x-ray and chemical examination. The results of this examination will be given in another article.

The enlargement and the histological picture of the parathyroid glands as well as the increase in the serum Ca and the drop in the inorganic blood P content point to the fact that the function of the parathyroid glands which expanded under the effect of the acidotic treatment increased.

In the evaluation of our experimental results, however, it is necessary to take into consideration that the blood Ca and P content is affected not only by the parathyroid glands but also by the hypophysis, pararenals and ovaries.

Koster and Geesink, as well as Vernetti and Gegerly observed in dogs, and Riddle in pigeons, and Hogben, Charles and Slome as well as Schapiro and Zwarenstein in frogs, the decrease in the blood Ca after hypophysectomy. Vernetti and Gegerly also determined a parathyroid atrophy and they attributed this to a lack of the hypophyseal function. Perla and Sandberg, as well as Collip, detected in hypophysectomized rats the negative trend of the Ca equilibrium, i.e. in those animals a Ca loss occurred. Putnam, Benedikt and Teel, and also Schapiro, observed in cats, Hoffmann and Anselmino observed in dogs, Pirolli in dogs, Pugsley and Anderson as well as Friedgood in guinea pigs and rats, Riddle in pigeons, that the blood-Ca with feeding of HVL extract the blood picture increased and at the same time there was an enlargement in the parathyroid glands. Anselmino and Hoffmann, as well as Berowitsch, found in dogs that the rise in the blood Ca with feeding of HVL extract did not occur when a removal of the parathyroid gland was performed in advance . This indicates that the hypophysis extract causes the blood Ca to increase by increasingthe function of the parathyroid glands. Other researchers indicated that the HVL extract also causes a Ca increase in the blood of animals which have been subjected to removal of the hypophysis (hypophysectomy) (Schapiro and Zwarenstein, Charles, as well as Hogben, Charles and Slome).

Bernabeo and Parra observed in one-sided (partial) extirpation of the pararenals a decrease in the blood Ca. Others, in contrast, found that after both-sided (bilateral) removal of the pararenals there was an increase in the Ca content of the blood (Hastings and Compere, Lucas, as well as Rogoff and Stewart, Suginato; Swingle and Pfiffner, Zwarenstein, Amantia). This agrees with the fact that Geene, as well as Sears, and furthermore Thaddea and Auersbach in certain groups of Addison's disease also found an increase in the blood serum Ca content. Leicher, Fiandaca , as well as Mirvish and Bosmann observed after administration of pararenal cortex hormone preparations a decrease in the blood serum Ca content.

Lorincz, Santha and Szabo found in healthy men and women as well as in women in the 2nd and 3rd months of pregnancy a small degree of decrease in the serum Ca under the effect of the administration over a period of ten days of desoxycorticosterone acetate. Thaddea was able to prevent the increase in the blood serum Ca in adrenalectomized animals and in Addison disease by administering pararenal cortex hormone. Fiandaca and Capizzi were also able to reduce the serum Ca content in Addison diseases by means of the"pararenal"cortex extract(translator: probably suprarenal cortex).

Other scientists observed after hypophysectomy a decrease in the inorganic P content in the blood (Anderson and Oestter, Jones and Shinowara, Li, Geschwind and Evans). Li, Geschwind and Evans found that the injection of the growth hormone of the hypophysis in hypophysectomized rats prevented the decrease in the inorganic serum P and even increased the P level above that of the control animals. Forsham and associates observed in normal humans after a large dose of ACTH (adrenocorticotropic hormone) an increase in the inorganic serum P content . Tornblom found a decrease in inorganic P in hypophysectomized rabbits, but after ACTH was administered an increase took place. Tornblom fed HVL extract to hypophysectomized and thyreoparathyroidectomized rabbits and he observed, along with an increase in the inorganic serum P a decrease in the serum Ca. Similar Ca and P changes occurred when a pararenal cortex extract was fed to such rabbits. Tornblom attributed this effect of the HVL extract partially to the growth hormone and also partially to the ACTH which comes into play on the course through the suprarenal glands .

As to the role of the ovaries, the observations indicate that the blood Ca increases considerably in birds during the laying time (Riddle and Reichart, Pfeifer and Gardner, Zondek and Marks) and this is taken to be a typical estrogen action. An increase in the blood C content could also be determined in mice, rats and dogs (Riddle and Reichart, Anselmino and Hoffmann). This increase does not occur, however, in the case of parathyroidectomized animals. This indicates that the estrogen exerts its action on its way through the parathyroid glands. In agreement with this, Dustin and Bullough observed in the parathyroid glands of estrogen-treated mice an increase in the mitotic cell division phenomena. In our opinion the follicle hormone also exerts its effect on the parathyroid glands on its way through the hypothalamus-hypophysis system.

In accordance with the data given above (in the medical literature) the Ca reduction and the P rise in the 1st week of the acidotic treatment could be explained by the increased function of the HVL. At this time the ACTH forms to a greater extent and through the increase in the function of the suprarenal cortex this causes the rise in P and the drop in Ca. This agrees well with the earlier finding of Fazekas, namely that under the effect of acidotic treatment not only do the parathyroid glands become larger but also the ovaries, the suprarenal glands and the hypophysis and they cause an increased

function. It appears that in the 1st week of treatment the parathyreotropic hormone is still not increased to the amount which would correspond to the increased demands and therefore a transition hypo function of the parathyroid glands occurs at that time (Ca decrease, P increase). In the 2nd and 3rd weeks of the acidotic treatment, on the other hand, and even later for two weeks after the treatment has been discontinued, there is an increase in Ca but a decrease in P, which indicates that the increased parathyreotropic hormone formation of the hypophysis causes at this time an increase in the function of the parathyroid glands and later even an enlargement, as was the case for Anselmino et al. after administering parathyreotrophic hormone. Some researchers hold that the existence of the parathyreotrophic hormone is not a proven fact. The explanation of this problem, however, would go beyond the scope of our work and therefore we would not like to become concerned with it at this time. Our results lead us to hope that perhaps the reduced function of the parathyroid glands can be made normal and that the pathological conditions which are caused by the reduced function can be eliminated.

SUMMARY

1. The author treated 72 male and female rabbits (in groups of 6 animals each) twice a day with acidotis-producing compounds for a period of time varying from 5 to 26 months in length. A period of three weeks of treatment was always followed by a pause in the treatment lasting 2 weeks. Afterwards, however, the treatment was continued in the same way. The Ca of the blood serum and the inorganic P content of the total blood were determined weekly.

2. After the first week of treatment in the 1st treatment period the serum Ca decreased add the inorganic P of the blood increased, however. In the 2nd and 3rd weeks of the treatment, on the other hand, there occurred a gradual increase in the serum Ca and a reduction in the inorganic P. During the treatment pause the serum Ca decreased gradually in comparison with the values of the 3-week treatment and even after the two-week pause its value was higher than the normal initial value. The inorganic P content of the blood increased once again during the first treatment pause above the normal initial value.

3. During the course of the next treatment period the serum Ca increased already during the first week of treatment and still more during the second and third weeks. The inorganic P content, however, decreased. The Ca increase and the P decrease were also noticed during the treatment pauses, although to a smaller extent than during the time of the treatment. The serum Ca turned out to be greater and the inorganic P content of the blood smaller, however, than before the treatment.

4. In agreement with our earlier experiments, there was a 160 to 277% enlargement of the parathyroid glands of the animals. Histologically there was an enrichment in blood, an increase and enlargement of the light main cells as well as nucleus division phenomena, i.e. the signs of a hyperfunction.

5. Since the Ca and P metabolism of the organism is primarily controlled by the functioning of the parathyroid glands, the experiments carried out by the author indicate that the function of the parathyroid glands at the time of the Ca decrease and P increase in the first week of treatmentdecreases temporarily. During the course of the further treatment at the time of the Ca increase and P decrease, however, it (the function) increases. The function of the parathyroid glands can therefore be affected in two ways by means of the acidotic treatment: 1. in the 1st week of treatment it can be reduced and from the second week on it can be increased. It is to be assumed that the function disturbances of the parathyroid glands could perhaps be favorably influenced through acidotic treatment.

figure on pp. 54 and 55

1= no.
2= chemicals which were used
3= no. of animals
4= mg % of Ca and P
5= pre-treatment
6= after
7= after
8= 1 2 3 weeks of treatment
9= 1 and 2 week treatment pause
10= after
11= after
12= after
13 = after
14= after
15= after
16= 1 2 3 weeks of treatment
17= 1 2 weeks of treatment pause
18= 1 2 3 weeks of treatment
19= 1 2 weeks of treatment pause
20= 1 2 3 weeks of treatment
21= 1 2 weeks of treatment pause
22= controls
23= 1 ammonium chloride
24= 2 ammonium sulphate
25= 3 sodium dihydr. phosphate
26= 4 hydrochloric acid
27= 5 lactic acid
28=6 acetic acid
29= 7 ammonium hydrophosphate
30= 8calcium chloride
31= 9 ammonium acetate
32= 10 ammonium lactate
33 = 11 Mixture of no.
1,4,5,7
8 and 9
34= Mixture of No.
1,4,5,7,
8 and 9

Fazekas-'54

Bib No. 113

Endokrinologie 32:45-57. 1954.

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Beeinflussung der Nebenschilddrüsenfunktion (Serum-Ca u. -P) durch azidotische Verbindungen

Von I. Gy. Fazekas

Mac Callum und Voegtlin wiesen bei Hunden nach Entfernung der Nebenschilddrüsen die Verminderung des Blut-Ca-Gehaltes nach. Greenwald sowie Hastings und Murray, Reed, Zachy und Payte beobachteten hingegen, daß der P-Gehalt des Blutes bei solchen Tieren zunimmt. Collip u. a. stellten fest, daß sich nach Einspritzung von Nebenschilddrüsenextrakten (Parathormon) der Ca-Gehalt des Blutserums so bei normalen wie bei parathyreoidektomisierten Hunden und Katzen vermehrt, der anorganische P-Gehalt sich aber vermindert. Es ist bekannt, daß bei Menschen, die an einer, durch Vergrößerung und gesteigerte Funktion der Nebenschilddrüsen bedingten Recklinghausen-Krankheit (Osteitis fibrosa generalisata) leiden, der Ca-Gehalt des Blutserums erheblich ansteigt, der anorganische P-Gehalt aber wesentlich abfällt. Gleiche Veränderungen des Serum-Ca- und P-Gehaltes wurden auch bei akutem Hyperparathyreoidis-

mus beobachtet (Olivier, Smith, Hanes). In verschiedenen Fällen des Hypoparathyreoidismus vermehrt sich das Serum-P auch bei Menschen neben Abnahme des Ca-Gehaltes des Blutserums. Die Nebenschilddrüsen spielen also in der Regulierung des Ca- und P-Stoffwechsels im Organismus eine Rolle ersten Ranges.

Fazekas stellte fest, daß sich die Nebenschilddrüsen von Kaninchen durch dauerhafte Verabreichung von azidotisch wirkenden Verbindungen um 171—294 % vergrößern. Der histologische Befund weist auf die gesteigerte Funktion der so vergrößerten Nebenschilddrüsen hin, das kann aber allein auf Grund des histologischen Bildes nicht entschieden werden. Um die Funktion der so vergrößerten Nebenschilddrüsen zu untersuchen, wurden neue Tierversuche angestellt. Da nach den Schrifttumsangaben zwischen dem Ca- und anorganischen P-Gehalt des Blutes sowie der Nebenschilddrüsenfunktion ein enger Zusammenhang besteht, war es anzunehmen, daß die systematische Untersuchung des Blut-Ca- und -P-Gehaltes bei Tieren, die mit azidotisch wirkenden, eine Nebenschilddrüsenhypertrophie verursachenden Verbindungen behandelt wurden, über die Funktion der Nebenschilddrüsen Auskunft geben werde.

In unseren Versuchen wurden 72 gleichartige (Chinchilla) und gleichaltrige (8—10 Monate alt) weibliche und männliche Kaninchen mit 2500 bis 3000 g Anfangsgewicht 5—26 Monate lang mit azidotisch wirkenden Verbindungen behandelt, 50 Tiere aber ohne Behandlung dienten zu gleicher Zeit als Kontrollen. Die Tiere wurden mit gleicher Menge von Hafer und trockenem Klee genährt. — Es wurden folgende Verbindungen verabreicht: Ammonium-chlorid, -sulfat, -hydrophosphat, -azetat, -laktat, Natriumdihydrophosphat, Acidum-hydrochloricum, -lacticum, -aceticum, Kalziumchlorid. — Mit jeder Verbindung wurden je 6 Tiere behandelt. Jedes Tier bekam 2 mal täglich 3 Wochen hindurch, in wöchentlich zunehmenden Dosen ,0,1—0,2 g/1 kg Körpergewicht von der entsprechenden Verbindung, in 50—80 ccm Trinkwasser gelöst. Danach folgte eine 2 wöchige behandlungsfreie Periode. So wiederholten sich Behandlung und Behandlungspause während 5—26 Monate. 6 Kaninchen wurden auf gleiche Art und Weise mit folgendem Verbindungsgemisch behandelt: 30 g Ammoniumchlorid, 5 g Ammoniumazetat, 5 g Ammoniumhydrophosphat, 10 g Kalziumchlorid, 5 g Acidum hydrochloricum, 5 g Acidum lacticum ad 1000 g aquae fontis. In 1 ccm dieser Lösung befindet sich 0,06 g Wirkstoffgemisch. Diese Tiere standen 16 Monate hindurch unter Behandlung. 6 andere Kaninchen wurden mit gleichem Verbindungsgemisch auf gleiche Weise, aber 26 Monate lang behandelt.

Bei den Versuchstieren wurden Ca-Gehalt des Blutserums und anorganischer P-Gehalt des Gesamtblutes vor, während und nach der Behandlung 2 Wochen lang wöchentlich bestimmt. Auch die Kontrollkaninchen wurden denselben Untersuchungen unterworfen. Der Ca-Gehalt wurde in 1 ccm Blutserum mit der oxydimetrischen Methode von Clark und Collip, der anorganische P-Gehalt des Blutes aber in 0,2 ccm Voll-

blut mit der kolorimetrischen Methode von Kuttner und Cohen mittels eines Stankoschen Elektrophotometers bestimmt. Der Mittelwert von je 2 parallel ausgeführten Bestimmungsgängen ergab immer das Resultat. Der Kürze wegen werden die Einzelangaben nicht mitgeteilt, sondern in beiliegender Tabelle lediglich die Mittelwerte der einzelnen Tiergruppen angeführt. Da bei der 11. Tiergruppe während der ersten 12 Monate die gleichen charakteristischen Serum-Ca- und -P-Veränderungen vorgefunden wurden als bei den 1.—10. Tiergruppen, werden diese Daten vereinzelt nicht besprochen, sondern nur die Ergebnisse der XI., XII. und XIII. Behandlungsperiode (13—16 Monate) angegeben. Bei der mit Verbindungsgemisch behandelten 12. Tiergruppe zeigte der Blutserum-Ca- und anorganische P-Gehalt des Blutes im Laufe der 18monatigen Behandlung Veränderungen gleichen Charakters wie bei den vorigen Gruppen. Bei der 12. Tiergruppe wurde nach 18monatiger Behandlung eine Pause von 3 Monaten eingeschaltet. Während dieser Zeit kehrte das Serum-Ca und anorganische P auf den normalen Ausgangswert zurück. Nachdem wurde die Behandlung der Tiere während 5 Monate (4 Behandlungsperioden) auf gleiche Art fortgesetzt.

Die ausführliche Auswertung der verschiedenen Ca- und P-Veränderungen soll hier wegbleiben, als Beispiel möchten wir nur die Auswertung der Behandlung mit Ammoniumsulfat sowie der 26monatigen Behandlung mit dem Verbindungsgemisch mitteilen.

Ammoniumsulfat-Behandlung

I. Behandlungsperiode. — Serum-Ca: Vor der Behandlung 14,92 mg-%. Nach 1wöchiger Behandlung 9,39 mg-%, was im Verhältnis zum normalen Anfangswert eine Senkung von 5,53 mg-% (37,06 %) bedeutet. Nach 2wöchiger Behandlung: 17,35 mg-%, d. h. um 2,45 mg-% (16,4 %), nach 3wöchiger Behandlung: 18,45 mg-%, d. i. um 3,53 mg-% (23,6 %) mehr. Nach 1wöchiger Behandlungspause 19,88 mg-%, nach 2wöchiger Pause 16,42 mg-%, was im Verhältnis zum normalen Anfangswert um 4,69 mg-% (31,9 %), bzw. 1,52 mg-% (10,0 %) mehr ist. — Blut-P: Vor der Behandlung 3,31 mg-%. Nach 1wöchiger Behandlung 4,13 mg-%, was einen Anwuchs von 0,82 mg-% (26,1 %) bedeutet. Nach 2wöchiger Behandlung 2,19 mg-%, nach 3wöchiger Behandlung 1,99 mg-%, d. h. um 1,12 mg-% (33,8 %) bzw. 1,32 mg-% (39,9 %) weniger als der normale Anfangswert. Nach 1wöchiger Behandlungspause 3,62 mg-%, nach 2wöchiger Pause 4,97 mg-%, d. i. um 1,63 mg-% (81,9 %) bzw. 2,98 mg-% (149,7 %) höher als nach 3wöchiger Behandlung und um 0,31 mg-% (9,3 %) bzw. 1,66 mg-% (50,1 %) höher als der normale Anfangswert.

II. Behandlungsperiode. — Serum-Ca: Nach 1wöchiger Behandlung 16,24 mg-%, nach 2wöchiger Behandlung 21,44 mg-%, nach 3 Wochen 23,11 mg-%, d. i. um 2,32 mg-% (15,55 %), um 6,52 mg-% (43,6 %) bzw. um 8,19 mg-% (54,9 %) mehr als der normale Anfangswert und um 5,02 mg-% (30,5 %) bzw. um 6,69 mg-% (40,7 %) höher als der

Wert nach 2wöchiger Pause der vorigen Behandlungsperiode. — Nach 1wöchiger Behandlungspause 20,77 mg-%, nach 2wöchiger Pause 16,26 mg-%, d. i. um 5,85 mg-% (39,2 %) bzw. 1,34 mg-% (8,9 %) höher als der normale Anfangswert. Das bedeutet im Verhältnis zum anfänglichen Behandlungswert einen Abstieg von 2,34 mg-% (10,1 %) bzw. 6,85 mg-% (29,9 %). — Blut-P: Nach 1wöchiger Behandlung 2,35 mg-%, nach 2wöchiger Behandlung 1,14 mg-%, nach 3wöchiger Behandlung 1,62 mg-%, was um 0,96 mg-% (29,0 %), 1,87 mg-% (56,5 %) bzw. 1,69 mg-% (51,0 %) niedriger als der normale Anfangswert. Nach einer Woche Pause 3,14 mg-%, nach 2wöchiger Behandlungspause 3,2 mg-%, d. h. um 0,17 mg-% (5,1 %) bzw. 0,11 mg-% (3,3 %) weniger als der normale Anfangswert.

III. Behandlungsperiode. — Serum-Ca: Nach 1wöchiger Behandlung 16,9 mg-%, nach 2 Wochen 20,51 mg-%, nach 3wöchiger Behandlung 22,66 mg-%. Diese Werte sind um 1,98 mg-% (13,3 %), 5,69 mg-% (37,6 %) bzw. 7,77 mg-% (51,8 %) höher als der normale Anfangswert. Nach 1wöchiger Behandlungspause 20,1 mg-%, nach 2wöchiger Pause 17,23 mg-%, was um 5,18 mg-% (35,3 %) bzw. 2,21 mg-% (14,8 %) höher ist als der normale Anfangswert, um 2,56 mg-% (11,2 %) bzw. 5,43 mg-% (23,9 %) hingegen niedriger als der Wert nach 3wöchiger Behandlung dieser Periode. — Blut-P: Nach 1wöchiger Behandlung 2,59 mg-%, nach 2wöchiger Behandlung 2,22 mg-%, nach 3 Wochen Behandlung 1,8 mg-%. Diese Werte sind um 0,62 mg-% (15,7 %), 1,09 mg-% (32,9 %) bzw. 1,51 mg-% (45,6 %) niedriger als der normale Anfangswert. — Nach 1wöchiger Behandlungspause 2,66 mg-%, nach 2wöchiger Pause 3,46 mg-%, d. i. um 0,65 mg-% (16,6 %) weniger bzw. um 0,15 mg-% (4,5 %) mehr als der normale Anfangswert. Der P-Wert kehrte also etwa aufs Normale zurück.

IV. Behandlungsperiode. — Serum-Ca: Nach 1wöchiger Behandlung 18,31 mg-%, nach 2wöchiger Behandlung 20,42 mg-%, nach 3wöchiger Behandlung 22,14 mg-%, d. i. um 3,39 mg-% (22,7 %) 5,50 mg-% (36,8 %) bzw. 7,22 mg-% (48,2 %) höher als der normale Anfangswert und um 1,08 mg-% (6,2 %), 3,19 mg-% (18,5 %) bzw. 4,91 mg-% (28,5 %) höher als der Wert nach der 2wöchigen Behandlungspause der vorigen Behandlungsperiode. — Nach 1wöchiger Behandlungspause [illegible],00 mg-%, d. i. um 3,68 mg-% (24,6 %) höher als der normale Anfangswert, aber um 3,54 mg-% (15,9 %) niedriger als nach der 3wöchigen Behandlung dieser Periode. — Blut-P: Nach 1wöchiger Behandlung [illegible] mg-%, nach 2wöchiger Behandlung 2,32 mg-%, nach 3wöchiger Behandlung 1,75 mg-%, was um 0,98 mg-% (29,6 %), 0,99 mg-% (29,9 %) bzw. 1,56 mg-% (47,1 %) weniger als der normale Anfangswert. — Nach [illegible]wöchiger Behandlungspause 2,63 mg-%, d. i. um 0,62 mg-% (45,2 %) [illegible] als der Wert nach 3wöchiger Behandlung dieser Periode, aber um [illegible] mg-% (20,5 %) weniger als der normale Anfangswert.

12. Behandlungsgruppe (Verbindungsgemisch)

XVIII. Behandlungsperiode. Serum-Ca: Vor der Behandlung 15,81 mg-%, nach 1wöchiger Behandlung 11,92 mg-%, was im Verhältnis zum normalen Anfangswert einen Abstieg von 3,89 mg-% (24,6 %) bedeutet. Diese Abnahme war aber nicht so hochgradig als bei Beginn der ersten Behandlung (37,2 %). Nach 2wöchiger Behandlung 17,81 mg-%, nach 3wöchiger Behandlung 22,86 mg-%, d. i. um 1,0 mg-% (6,3 %) bzw. 7,05 mg-% (44,59 %) mehr als der normale Anfangswert. Nach 1wöchiger Behandlungspause 20,27 mg-%, nach 2wöchiger Pause 16,35 mg-%, d. i. um 2,59 mg-% (11,3 %) bzw. 6,51 mg-% (29,3 %) weniger als nach 3wöchiger Behandlung dieser Periode, aber um 4,46 mg-% (28,2 %) bzw. 0,54 mg-% (3,4 %) mehr als der normale Anfangswert. — Blut-P: Vor der Behandlung 3,72 mg-%. Nach 1wöchiger Behandlung 4,58 mg-%, d. i. um 0,86 mg-% (23,1 %) höher als der normale Anfangswert. Nach 2wöchiger Behandlung 2,77 mg-%, nach 3wöchiger Behandlung 1,88 mg-%, d. i. um 0,95 mg-% (25,5 %) bzw. 1,84 mg-% (49,4 %) weniger als der normale Anfangswert. — Nach 1wöchiger Behandlungspause 2,87 mg-%, nach 2wöchiger Behandlungspause 3,07 mg-%, d. i. um 0,85 mg-% (22,8 %) bzw. 0,65 mg-% (17,4 %) weniger als der normale Anfangswert, aber um 0,99 mg-% (52,6 %) bzw. 1,19 mg-% (63,1 %) mehr als nach der 3wöchigen Behandlung dieser Periode.

XIX. Behandlungsperiode. — Serum-Ca: Nach 1wöchiger Behandlung 15,66 mg-%, d. i. fast gleich mit dem normalen Anfangswert. Ein Beweis dafür, daß der Organismus die Ca-vermindernde Wirkung der Behandlung schon abzuwehren vermag. Nach 2wöchiger Behandlung 19,84 mg-%, nach 3wöchiger Behandlung 22,63 mg-%, das im Verhältnis zum normalen Anfangswert eine Ca-Zunahme von 4,03 mg-% (23,4 %) bzw. 6,82 mg-% (43,1 %) bedeutet. — Nach 1wöchiger Behandlungspause 20,60 mg-%, nach 2wöchiger Pause 16,41 mg-%, was im Verhältnis zum Wert nach der 3wöchigen Behandlung dieser Periode einer Senkung von 2,03 mg-% (8,4 %) bzw. 6,22 mg-% (27,4 %) entspricht, aber um 4,79 mg-% (30,3 %) bzw. 60 mg-% (3,7 %) mehr beträgt als der normale Anfangswert. — Blut-P: Nach 1wöchiger Behandlung 3,23 mg-%, nach 2wöchiger Behandlung 2,44 mg-%, nach 3wöchiger Behandlung 2,30 mg-%, d. i. um 0,49 mg-% (13,1 %), 1,28 mg-% (34,4 %) bzw. 1,42 mg-% (38,1 %) weniger als der normale Anfangswert. — Nach einer Woche Pause 3,05 mg-%, nach 2wöchiger Behandlungspause 3,39 mg-%, d. i. um 0,67 mg-% (18,0 %) bzw. 0,33 mg-% (8,8 %) weniger als der normale Anfangswert, aber um 0,75 mg-% (32,6 %) bzw. 1,09 mg-% (47,3 %) mehr als nach der 3wöchigen Behandlung dieser Periode.

XX. Behandlungsperiode. — Serum-Ca: Nach 1wöchiger Behandlung 16,93 mg-%, nach 2wöchiger Behandlung 19,88 mg-%, nach 3wöchiger Behandlung 21,99 mg-%, d. h. um 1,12 mg-% (7,0 %), 5,07 mg-% (26,3 %) bzw. 6,18 mg-% (39,0 %) höher als der normale An-

fangswert. — Nach 1wöchiger Behandlungspause 20,06 mg-%, nach 2wöchiger Pause 17,21 mg-%, d. i. um 1,93 mg-% (8,7 %) bzw. 4,78 mg-% (21,4 %) weniger als nach der 3wöchigen Behandlung dieser Periode, aber um 1,25 mg-% (26,8 %) bzw. 1,40 mg-% (9,4 %) mehr als der normale Anfangswert. — Blut-P: Nach 1wöchiger Behandlung 2,61 mg-%, nach 2wöchiger Behandlung 2,28 mg-%, nach 3wöchiger Behandlung 2,10 mg-%, d. i. um 1,11 mg-% (29,8 %), 1,51 mg-% (41,3 %) bzw. 1,62 mg-% (43,5 %) weniger als der normale Anfangswert. — Nach 1wöchiger Behandlungspause 2,53 mg-%, nach 2wöchiger Pause 2,75 mg-%, d. i. um 1,19 mg-% (31,9 %) bzw. 0,97 mg-% (26,0 %) weniger als der normale Anfangswert, aber um 0,43 mg-% (20,4 %) bzw. 0,65 mg-% (30,9 %) mehr als nach der 3wöchigen Behandlung dieser Behandlungsperiode.

XXI. Behandlungsperiode. — Serum-Ca: Nach 1wöchiger Behandlung 17,22 mg-%, gleicher Wert als nach 2wöchiger Behandlungspause der vorigen Periode, was auch bedeutet, daß der Organismus die Ca-vermindernde Wirkung der 1wöchigen Behandlung zu kompensieren vermag. Nach 2wöchiger Behandlung 19,38 mg-%, nach 3wöchiger Behandlung 22,05 mg-%, was um 3,57 mg-% (28,9 %) bzw. 6,24 mg-% (39,4 %) höher ist als der normale Anfangswert. — Nach 1wöchiger Behandlungspause 18,94 mg-%, d. i. um 3,11 mg-% (19,7 %) weniger als nach der 3wöchigen Behandlung dieser Periode, aber um 3,13 mg-% (19,8 %) mehr als der normale Anfangswert. — Blut-P: Nach 1wöchiger Behandlung 2,72 mg-%, nach 2wöchiger Behandlung 2,28 mg-%, nach 3wöchiger Behandlung 1,78 mg-%, d. i. um 1,0 mg-% (26,5 %), 1,44 mg-% (38,6 %) bzw. 1,94 mg-% (52,1 %) weniger als der normale Anfangswert. Nach 1wöchiger Behandlungspause 2,65 mg-%, d. i. um 1,07 mg-% (28,7 %) weniger als der normale Anfangswert, aber um 0,87 mg-% (49,4 %) höher als nach 3wöchiger Behandlung dieser Periode.

Auch die Untersuchungen mit den übrigen Verbindungen zeigten gleiche Ca- und P-Veränderungen. Bei den Kontrollkaninchen konnten aber keine ähnlichen und gleichmäßigen Veränderungen des Serum-Ca und anorganischen P festgestellt werden.

Die Ergebnisse zeigen also, daß unter dem Einfluß der verwendeten azidotischen Verbindungen, nach der erstwöchigen Behandlung der ersten Periode, das Serum-Ca wesentlich (24—37 %) unter den Anfangswert sinkt, das Blut-P aber zu gleicher Zeit 20—42 % über den normalen Wert ansteigt. Das steht im Einklang mit unserer früheren Feststellung, daß unter dem Einfluß einer Dosis von NH_4OH bzw. NaOH das Serum-Ca abnimmt, das Blut-P aber zunimmt. In der 2. Behandlungswoche tritt eine Steigerung des Serum-Ca (10—35 %) und ein Absinken des Blut-P (11—36 %) auf, was nach 3wöchiger Behandlung sein Maximum erreicht (Ca: 20—51 %; P: 24—50 %). Nach der 1- und 2wöchigen Behandlungspause nimmt das Serum-Ca im Verhältnis zum Wert der 3wöchigen Behandlung allmählich ab, aber bleibt meistens auch noch nach 2 Wochen höher (7—21 %) als der normale Anfangswert, das Blut-P erhebt sich hin-

gegen wiederholt über das normale (50—67 %). Während der folgenden Behandlungsperioden sinkt das Serum-Ca nach der erstwöchigen Behandlung niemals unter den normalen Wert, sondern bleibt auch zu dieser Zeit um 12—22 % höher, das Blut-P aber fällt wesentlich (20—30 %) unter den Normalwert. In den 2. und 3. Behandlungswochen ist so die Zunahme des Serum-Ca (50—65 %) wie die Abnahme des Blut-P (30—56 %) noch deutlicher als zur selben Zeit der ersten Behandlungsperiode. Im Laufe der 2wöchigen Behandlungspausen nimmt das Serum-Ca allmählich ab, das Blut-P aber zu, im Verhältnis zum Wert der 3wöchigen Behandlung. Das Serum-Ca ist aber auch noch dann höher, das anorganische P aber niedriger als der normale Anfangswert, d. h. das Serum-Ca stellt sich auf höher, das Blut-P aber auf niedriger ein, als es vor dem Behandlungsbeginn stand.

Bei den mit dem Verbindungsgemisch 16 Monate lang behandelten Kaninchen und bei denen, die nach einer 18monatigen Behandlung und darauffolgenden 3monatigen Behandlungspause aufs neue behandelt wurden (insgesamt 26 Monate), konnten Ca- und P-Veränderungen gleichen Charakters beobachtet werden.

Nach Tötung der Versuchstiere wurde eine 160,8—277,7 %ige Nebenschilddrüsenvergrößerung (Mittelwerte!) vorgefunden. In den Nebenschilddrüsen konnten histologisch, im Einklang mit unseren früheren Untersuchungen, Blutfülle, Vermehrung und Vergrößerung der hellen Zellen, Kernteilungserscheinungen, also die Anzeichen einer Hyperfunktion beobachtet werden.

Andere Verfasser verzeichneten gleichfalls eine Nebenschilddrüsenvergrößerung bei dauerhafter Verabreichung von Ammonium chlorid, Glukose und Hydroxylamin (Azidose). Die Veränderung des Blut-Ca und -P untersuchten sie aber nicht und so konnten sie auch die Funktionssteigerung der vergrößerten Nebenschilddrüsen nicht beweisen. Es ist bekannt, daß in gewissen Fällen der chronischen Niereninsuffizienz eine sog. sekundäre Hyperplasie und gesteigerte Funktion der Nebenschilddrüsen entsteht, was dann charakteristische Knochenveränderungen verursachen kann. Das Knochensystem unserer Versuchstiere wurde, nach Ergänzung mit neueren Versuchen, einer histologischen, röntgenologischen und chemischen Untersuchung unterzogen. Über das Ergebnis wird in einer anderen Arbeit berichtet.

Die Vergrößerung und das histologische Bild der Nebenschilddrüsen sowie der Anstieg des Serum-Ca und das Sinken des anorganischen Blut-P-Gehaltes weisen darauf hin, daß sich die Funktion der unter dem Einfluß der azidotischen Behandlung vergrößerten Nebenschilddrüsen steigerte.

Bei der Auswertung unserer Versuchsergebnisse muß aber in Betracht gezogen werden, daß der Blut-Ca- und -P-Gehalt nicht nur durch die Nebenschilddrüsen, sondern auch durch die Hypophyse, Nebennieren und Ovarien beeinflußt werden.

Köster und Geesink sowie Vernetti, Gegerly beobachteten an Hunden. Riddle an Tauben. Hogben, Charles und Slome sowie Schapiro und Zwarenstein an Fröschen die Abnahme des Blut-Ca nach Hypophysektomie. Vernetti, Gegerly stellten dabei noch eine Nebenschilddrüsenatrophie fest und führten diese auf den Mangel der Hypophysenfunktion zurück. Perla und Sandberg sowie Collip wies bei hypophysektomisierten Ratten das Negativwerden des Ca-Gleichgewichtes nach, d. i. bei solchen Tieren tritt ein Ca-Verlust auf. Putnam, Benedikt und Teel, ferner Schapiro beobachteten an Katzen, Hoffmann und Anselmino an Hunden, Pirolli an Kaninchen, Pugsley und Anderson sowie Friedgood an Meerschweinchen und Ratten, Riddle an Tauben, daß sich das Blut-Ca bei Verabreichung von HVL-Extrakt erhöht und auch die Nebenschilddrüsen sich zu gleicher Zeit vergrößern. Anselmino und Hoffmann sowie Berowitsch fanden bei Hunden, daß der Anstieg des Blut-Ca bei Verabreichung von HVL-Extrakt nicht eintrifft, wenn vorher an den Tieren eine Nebenschilddrüsenentfernung durchgeführt worden ist. Das weist darauf hin, daß das Hypophysenextrakt den Anstieg des Blut-Ca durch die Steigerung der Nebenschilddrüsenfunktion hervorruft. Andere Forscher wiesen nach, daß das HVL-Extrakt auch bei hypophysektomierten Tieren eine Ca-Steigerung des Blutes hervorruft (Schapiro u. Zwarenstein, Charles sowie Hogben, Charles u. Slome).

Bernabeo und Parra beobachteten nach einseitiger Nebennierenexstirpation die Abnahme des Blut-Ca, andere stellten hingegen fest, daß nach beiderseitiger Entfernung der Nebennieren der Ca-Gehalt des Blutserums ansteigt (Hastings und Compere, Lucas sowie Rogoff u. Stewart, Suginato; Swingle u. Pfiffner, Zwarenstein, Amantia). Damit steht in Einklang, daß Geene sowie Sears, ferner Thaddea und Auersbach bei gewissen Gruppen der Addison-Kranken gleichfalls eine Steigerung des Blutserum-Ca-Gehaltes vorfanden. Leicher, Fiandaca sowie Mirvish und Bosmann beobachteten nach Darreichung von Nebennierenrindenhormonpräparaten das Sinken des Blutserum-Ca-Gehaltes. Lörincz, Sántha und Szabó fanden bei gesunden Männern und Frauen sowie bei Frauen im 2.—3. Schwangerschaftsmonat eine kleingradige Abnahme des Serum-Ca unter dem Einfluß des 10 Tage lang verabreichten Desoxycorticosteronacetat. Thaddea gelang bei adrenalektomisierten Tieren und bei Addison-Kranken den Anstieg des Blutserum-Ca durch Verabreichung von Nebennierenrindenhormon zu verhindern. Fiandaca und Capizzi konnten gleichfalls den Serum-Ca-Gehalt bei Addison-Kranken durch Nebennierenrindenextrakt vermindern.

Andere Forscher beobachteten nach einer Hypophysektomie die Abnahme des anorganischen P-Gehaltes im Blut (Anderson u. Oestter, Jones u. Shinowara, Li, Geschwind u. Evans). Li, Geschwind u. Evans wiesen nach, daß die Einspritzung des Wachstumshormons der Hypophyse bei hypophysektomierten Ratten das Absinken des anorga-

nischen Serum-P verhindert, sogar das P-Niveau über das der Kontrollen ansteigt. Forsham u. Mitarbeiter beobachteten bei normalen Menschen nach großer ACTH-Dosis den Anstieg des anorganischen Serum-P-Gehaltes. Törnblom stellte bei hypophysektomisierten Kaninchen die Abnahme des anorganischen P des Serums fest, das aber nach ACTH-Darreichung anstieg. Törnblom verabreichte hypophysektomisierten und thyreoparathyreoidektomisierten Kaninchen HVL-Extrakt und beobachtete neben Anstieg des anorganischen Serum-P die Abnahme des Serum-Ca. Gleiche Ca- und P-Veränderungen zeigten sich, wenn solchen Kaninchen ein Nebennierenrindenextrakt gegeben wurde. Törnblom führt diese Wirkung des HVL-Extraktes teils auf das Wachstumshormon, teils aber auf das ACTH zurück, das auf dem Wege über die Nebennieren zur Geltung kommt.

Auf die Rolle der Ovarien weisen die Beobachtungen hin, nach denen sich das Blut-Ca bei Vögeln während der Legeperiode stark vermehrt (Riddle u. Reichart, Pfeifer u. Gardner, Zondek u. Marks), was als typische Östrogenwirkung aufgefaßt wird. Nach Verabreichung von Östrogen konnte auch bei Mäusen, Ratten und Hunden eine Zunahme des Blut-Ca-Gehaltes festgestellt werden (Riddle u. Reichart, Anselmino u. Hoffmann), dieser Anstieg bleibt aber bei parathyreoidektomisierten Tieren weg. Diese Tatsache weist darauf hin, daß das Östrogen seine Wirkung auf dem Wege über die Nebenschilddrüsen ausübt. In Einklang damit beobachteten Dustin und Bullough in den Nebenschilddrüsen von östrogenbehandelten Mäusen die Vermehrung der mitotischen Zellteilungserscheinungen. Unserer Ansicht nach übt auch das Follikelhormon seinen Einfluß auf die Nebenschilddrüsen auf dem Wege über das Hypothalamus-Hypophysensystem aus.

Im Spiegel der obigen Schrifttumsangaben könnte das Ca-Sinken und der P-Anstieg in der 1. Woche der azidotischen Behandlung mit der gesteigerten Funktion des HVL erklärt werden. Zu dieser Zeit bildet sich das ACTH nämlich in größerem Maße und das ruft durch die Funktionssteigerung der Nebennierenrinde den P-Anstieg und die Ca-Abnahme hervor. Das ist mit der früheren Behauptung (Fazekas) gut zu vereinbaren, daß nämlich unter dem Einfluß azidotischer Behandlung nicht nur die Nebenschilddrüsen, sondern auch die Ovarien, Nebennierenrinde und Hypophyse größer werden und eine gesteigerte Funktion entfalten. Es hat den Anschein, daß das Parathyreotrophormon in der 1. Behandlungswoche von der Hypophyse noch nicht in den gesteigerten Ansprüchen entsprechender Menge gebildet wird, weshalb eine Übergangshypofunktion der Nebenschilddrüsen zu dieser Zeit entsteht (Ca-Abnahme, P-Anstieg). In der 2. und 3. Woche der azidotischen Behandlung hingegen, sogar auch später während 2 Wochen nach Abbrechung der Behandlung, nimmt das Ca zu, das P aber ab, was darauf hinweist, daß die gesteigerte Parathyreotrophormonbildung der Hypophyse zu dieser Zeit eine Funktionssteigerung der Nebenschilddrüsen, später auch ihre Vergrößerung hervorruft, wie es bei

Anselmino u. Mitarbeiter nach Verabreichung von Parathyreotrophormon der Fall war. (Einige Verfasser halten die Existenz des Parathyreotrophormons für nicht bewiesen; die Erörterung dieser Frage würde aber den Rahmen unserer Arbeit übertreten, weshalb wir uns damit nicht zu beschäftigen wünschen.) Unsere Ergebnisse lassen hoffen, daß gegebenenfalls die verminderte Funktion der Nebenschilddrüsen normal gemacht und die durch verminderte Funktion bedingten pathologischen Umstände aufgehoben werden können.

Zusammenfassung

1. Verfasser behandelte 72 männliche und weibliche Kaninchen (in Gruppen mit je 6 Tieren) 2täglich mit azidotisch wirkenden Verbindungen 5—26 Monate lang. Nach 3wöchiger Behandlung wurde immer eine 2wöchige behandlungsfreie Periode eingeschaltet, nachher aber die Behandlung auf gleiche Weise fortgesetzt. Das Ca des Blutserums und der anorganische P-Gehalt des Gesamtblutes wurden wöchentlich festgestellt.

Nr.	Verwendete Chemikalien	Zahl der Tiere	mg-% Ca und P	Vor Behandlung	I nach 1 wöchiger Behandlung	I nach 2 wöchiger Behandlung	I nach 3 wöchiger Behandlung	nach 1 wöchiger Behandlungspause	nach 2 wöchiger Behandlungspause
	Kontrollen	50	Ca	15,60	15,40	15,55	15,62	15,70	15,80
			P	3,65	3,65	3,50	3,70	3,60	3,72
1	Ammonium chlorid	6	Ca	14,82	9,31	16,39	17,83	19,93	17,29
			P	3,07	3,73	2,73	2,33	4,28	5,14
2	Ammonium sulfat	6	Ca	14,92	9,39	17,37	18,45	19,88	16,42
			P	3,31	4,13	2,19	1,99	3,62	4,67
3	Natr. dihydr. phosphat	6	Ca	14,19	10,27	18,78	19,57	20,41	15,55
			P	3,55	4,27	1,92	1,85	3,56	4,64
4	Acid. hydrochlor.	6	Ca	16,52	12,42	22,22	24,96	21,27	18,92
			P	3,80	5,27	2,81	2,03	2,78	3,21
5	Acid. lacticum	6	Ca	15,40	10,90	20,10	23,10	21,17	18,70
			P	3,82	5,43	2,55	1,92	2,68	3,09
6	Acid. aceticum	6	Ca	15,48	10,61	16,78	17,56	21,27	18,53
			P	3,09	4,55	2,60	2,22	4,16	4,91
7	Ammon. hydrophosphat	6	Ca	15,08	9,53	16,84	17,97	18,78	16,22
			P	3,23	3,95	1,94	1,88	3,12	5,36
8	Calcium chlorid	6	Ca	14,18	8,85	15,57	17,40	20,31	17,42
			P	3,19	3,98	2,92	2,46	4,32	5,28
9	Ammon. acetat.	6	Ca	15,83	9,96	17,05	18,14	20,09	16,51
			P	3,09	3,93	2,85	2,54	4,85	5,25
10	Ammon. lactat	6	Ca	14,95	9,79	18,52	19,50	19,62	15,50
			P	3,19	4,65	2,01	1,96	3,97	4,77
11	Gemisch von Nr. 1, 4, 5, 7, 8 und 9	6	Ca	16,19	18,60	22,36	XI 25,18	21,99	19,45
			P	3,86	5,06	3,33	2,60	3,11	3,71
12	Gemisch von Nr. 1, 4, 5, 7, 8 und 9	6	Ca	15,81	11,92	17,81	XVIII 22,86	20,27	16,35
			P	3,72	4,78	2,77	1,88	2,87	3,07

2. Nach 1wöchiger Behandlung der 1. Behandlungsperiode nahm das Serum-Ca ab, das anorganische P des Blutes aber zu. In der 2. und 3. Woche der Behandlung hingegen vermehrte sich allmählich das Serum-Ca und verminderte sich das anorganische P. Während der Behandlungspause sank das Serum-Ca allmählich im Verhältnis zu den Werten der 3wöchigen Behandlung, auch nach der 2wöchigen Pause war sein Wert höher als der normale Anfangswert. Der anorganische P-Gehalt des Blutes erhob sich während der ersten Behandlungspause wieder über den normalen Anfangswert.

3. Im Laufe der weiteren Behandlungsperiode vermehrte sich das Serum-Ca auch schon während der erstwöchigen Behandlung, noch mehr während der 2. und 3. Woche, der anorganische P-Gehalt nahm aber ab. Die Ca-Vermehrung und die P-Herabsetzung war auch während der Behandlungspausen festzustellen, obwohl in kleinerem Maße als zur Zeit der Behandlung. Das Serum-Ca stellte sich also höher, der anorganische P-Gehalt des Blutes aber niedriger ein als vor der Behandlung.

Behandlungsperiode:																	
II					III						IV						
nach			nach		nach			nach			nach			nach			
1	2	3	1	2	1	2	3	1	2		1	2	3	1	2		
wöchiger Behandlung			wöchiger Behandlungspause		wöchiger Behandlung			wöchiger Behandlungspause			wöchiger Behandlung			wöchiger Behandlungspause			
15,60	15,82	15,51	15,42	15,70	15,45	15,80	15,50	15,81	16,00		15,70	15,62	15,80	15,45	15,90		
3,80	3,73	3,80	3,70	3,65	3,50	3,55	3,40	3,60	3,62		3,55	3,63	3,70	3,73	3,69		
16,70	21,41	24,02	21,33	16,17	16,78	21,12	22,71	20,26	17,44		17,68	20,44	22,31	19,37	18,[illegible]		
2,43	1,41	1,89	3,01	3,22	2,84	2,34	2,18	2,70	3,44		2,50	2,23	1,77	2,82	3,20		
16,24	21,44	23,11	20,77	16,26	16,90	20,51	22,66	20,10	17,23		18,31	20,42	22,14	18,60	17,90		
2,85	1,44	1,62	3,14	3,20	2,59	2,22	1,80	2,66	3,46		2,33	2,32	,75	2,63	2,81		
15,04	22,78	23,41	21,19	17,15	17,65	21,34	22,27	20,31	17,37		18,39	20,67	22,16	18,67	18,[illegible]		
2,54	1,57	1,34	2,81	3,07	2,53	2,16	1,96	2,78	3,81		2,20	2,25	1,69	2,72	2,90		
19,02	21,75	24,02	21,60	20,02	19,17	22,02	23,97	21,30	20,30		19,30	22,37	24,70	21,70	20,10		
3,43	2,69	2,05	2,44	2,91	2,50	2,26	1,94	2,50	3,19		2,67	2,38	2,01	2,41	2,85		
17,67	20,65	23,00	20,27	19,10	18,40	21,40	23,07	20,92	19,40		18,50	21,20	23,70	21,20	19,80		
3,36	2,68	2,45	2,55	2,81	2,64	2,22	2,00	2,44	3,14		2,73	2,45	2,12	2,48	2,82		
16,59	22,20	23,36	20,17	15,14	16,45	21,34	22,57	20,62	17,34		17,87	19,86	22,76	19,37	13,20		
2,24	1,27	1,50	2,76	3,20	2,75	2,61	2,42	2,87	3,73		2,47	2,41	1,73	2,90	3,12		
15,39	22,55	22,97	21,03	15,42	16,43	21,34	22,32	20,65	17,62		17,92	19,63	21,36	18,62	18,20		
2,27	1,50	1,37	3,31	3,22	2,79	2,35	2,15	2,59	3,49		2,16	2,50	1,73	2,44	2,90		
16,80	21,48	25,24	22,60	17,18	17,60	22,75	23,80	21,35	18,35		18,10	21,30	22,35	21,25	20,00		
2,60	1,58	1,25	3,30	3,10	2,80	2,35	2,00	2,75	3,40		2,45	2,10	1,82	2,30	2,85		
16,99	21,89	23,16	21,37	16,34	16,68	22,01	22,91	19,70	16,74		17,37	19,80	22,21	19,10	18,60		
3,59	1,18	1,37	3,10	3,27	2,74	2,25	2,25	2,68	3,57		2,58	2,20	1,61	2,77	2,80		
14,67	21,68	22,40	21,19	17,21	17,71	19,99	22,68	19,83	17,19		17,22	21,60	22,31	17,96	17,60		
2,75	1,36	1,38	2,72	2,84	2,58	2,25	2,01	2,68	4,42		2,32	2,17	1,73	2,69	2,85		
		XII					XIII										
20,31	21,71	23,95	21,24	19,44	19,81	21,08	21,92	21,73	19,78								
3,40	2,80	2,30	3,11	3,74	3,01	2,62	2,10	2,62	2,87								
		XIX					XX						XXI				
18,65	19,84	22,63	20,60	16,41	16,93	19,88	21,99	20,06	17,21		17,22	19,38	22,05	18,94	18,50		
3,28	2,41	2,30	3,05	3,39	2,61	2,28	2,10	2,68	2,75		2,72	2,28	1,78	2,65	2,70		

4. An den Nebenschilddrüsen der Tiere zeigten sich — im Einklang mit unseren früheren Untersuchungen — eine 160—277 %ige Vergrößerung, histologisch ein Blutreichtum, Vermehrung und Vergrößerung der hellen Hauptzellen sowie Kernteilungserscheinungen, d. i. Anzeichen einer Hyperfunktion.

5. Da der Ca- und P-Stoffwechsel des Organismus hauptsächlich durch die Funktion der Nebenschilddrüsen reguliert wird, weisen die Versuche des Verfassers darauf hin, daß sich die Funktion der Nebenschilddrüsen zur Zeit der Ca-Abnahme und P-Vermehrung in der 1. Behandlungswoche übergänglich vermindert, im Laufe der weiteren Behandlung zur Zeit der Ca-Vermehrung und P-Abnahme aber steigert. Die Funktion der Nebenschilddrüsen kann also durch azidotische Behandlung in zwei Richtungen beeinflußt werden: in der 1. Behandlungswoche kann sie vermindert, von der 2. Woche ab gesteigert werden. Es ist anzunehmen, daß die Funktionsstörungen der Nebenschilddrüsen durch azidotische Behandlung vielleicht günstig beeinflußt werden könnten.

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Virchow's Archive, Vol. 324, p. 531 to 542 (1954)

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Fazekas-'54
Bib No. 114

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With 3 text illustrations.

(Received on July 1953).

Reports on the enlargement of parathyroid in man were published first by ERDHEIM (1903) and subsequently by CLAUDE and BAUDOUIN, REINHARD and CREUZFELD, BALLET and LAVASTINE, JOSEPHSON, CUSHING and DAVIDOFF, HOFF and others in conjunction with basophil adenomas of the hypophysis front lobe (HVL), as well as by LLOYD in connection with the primary cell adenomas of the hypophysis. In 1904 RECKLINGHAUSEN prepared a pathography of osteosis fibrosa generalisata which develops under the influence of the increased adrenal gland function due to adenomatous or hyperplastic causes. The correlation between the modification or hyperfunction respectively, of the bones and adrenal glands was verified first by MANDL (1926) and later by others on the basis of the fact that patients recovered after the surgical removal of their adenomatous or hyperplastic parathyroid. The so-called secondary hyperplasia of the parathyroid can occur as a compensating reaction in case of an increased para-hormone need of the organism, for example in case of bone metastases of a carcinoma, in case of myeloma multiplex, rachitis, CUSHING's disease, acute kidney diseases (CAMERON, VERZAR), in case of gravidity (ARVAY), partial or total kidney extirpation, as well as in case of a phosphorus content increase of the diet (GOURNOT).

An enlargement of the parathyroid was observed by ANSELMINO, HOFFMANN and HEROLD after the application during several days of HVL-extract in rats, later in other animals; HERTZ and KRANES observed the same in rabbits, HAM and HAIS in dogs. They assumed, on the basis of these findings, that HVL forms a hormone which stimulates the parathyroid This coincides fully with the findings of SMITH in rats, KOSTER, HOUSSAY and BIASOTTI and those of HOUSSAY and SAMMARTINO as well as VERNETTI who found in dogs after the extirpation of the hypophysis an atrophy and a degenerative atrophy of the parathyroid. LIVON and PEYRON, as well as ASCHNER (in dogs) and subsequnetly COLLIP (in rats), on the other hand, found no parathyroid atrophy after the removal of the hypophysis. Using rabbits, CATTANEO did not obtain any enlargement of the parathyroid with the hypophysis extract. In 1949 TORNBLOM found a high-grade enlargement of the parathyroid in rabbits after the administration of large quantities of sodium and ammonium phosphate, simultaneously with a calcium-deficient diet during 12 weeks.

investigations carried out to date clearly indicate that the increased or reduced function of the parathyroid causes various pathological conditions through the radical changes brought about in the calcium- and phosphorus metabolism of the organism. It would therefore be desirable to be able to influence the parathyroid function with a method which would eliminate diseases resulting from decreased function by re-establishing said function.

It was found during earlier investigations (FAZEKAS) that the continued administration of compounds with acidotic effect can cause the following in rabbits, geese,. . . (illegible word), swine and in goats: hypertrophy and increased function of the adrenal gland core, enlargement of the ovaries and uterus, follicle. maturity (illeg) and follicle hemorrhage, formation of yellow bodies ~~corpuscles~~, cyclic changes of the mucous membrane of the uterus tubes, obesity, enlargement of the breasts and milk secretion , as well as enlargement of the hypophysis and multiplication of the basophil cells of the HVL. The following investigations are intended to show a possibility for chemical action on the parathyroid.

The compounds listed on the Table were used for the treatment of the animals. The parathyroid of 362 treated rabbits (including animals from previous tests as well) were examined. Size, weight and histological . . (illeg.) were compared with the corresponding data of the control animals. 50 similar rabbits of the same age and weight were used for control. -- The survey table shows that the parathyroid of the untreated control animals 6 to 14 had an average weight of 9,2 mg; the parathyroid of the treated rabbits, on the other hand, showed a considerable weight increase.

1. <u>Ammonium-hyroxide</u> was used for treating 96 female and 64 male animals during 4 - 5 - 16 months. Each animal received twice daily 50 to 80 cm^3 ½% of NH_4OH through the stomach probe in gradually increasing doses. 3 weeks of therapy were always followed by a period of one week without therapy. The body weight of the animals increased gradually during treatment; at the time when they were killed, it amounted to 3100 to 4400 g. - The adrenal glands weighed 17 to 39 mg, 26 mg on the average, which is 16.8 mg, i.e. 182.6% more as compared to the weight of the adrenal glands of the controls. According to the statistical calculations the significant difference amounted to 10.84; therefore the weight increase of the parathyroid is specific.

2. <u>Ammonium-chloride</u> was used for treating 50 female and 30 male rabbits during 3 - 5 - and 16 months. Every second day the animals received 0,1 to 0,2 g/kg of NH_4Cl dissolved in 100 cm^3 of drinking water, in gradually increasing doses. A 3-week treatment was always followed by an interruption of 1 week . During the treatment period the weight of the animals increased gradually; at the time when they were killed, the weight with air embolism amounted to 3500 to 4500 g. The weight of the parathyroid was 16,5 - 57,5 mg, i.e. 25,3 mg more than for the controls; this corresponds to a weight increase of 274,6%. The significance was found for 9,03.

Table 1. Weight data of the parathyroid for the treated rabbits and for the controls.

Tabelle 1. *Gewichtsangaben der Nebenschilddrüsen bei behandelten Kaninchen und Kontrollen.*

		1 Zahl der Tiere	2 Gewicht der Nebenschilddrüsen in mg			6 Gewichtszunahme der Nebenschilddrüsen im Mittelwert		s. D.
			3 minimal	4 maximal	5 Mittelwert	%	mg	
	Kontrollen	50	6	14	9,2	–	–	–
1	Ammoniumhydroxyd	160	17,0	39,0	26,0	16,8	182,6	10,84
2	Ammoniumchlorid	80	16,5	57,5	34,5	25,3	274,6	9,03
3	Ammoniumsulfat	10	15,0	33,0	27,5	18,3	198,9	7,04
4	Ammoniumcarbonat	14	15,0	40,0	25,0	15,8	171,9	6,86
5	Natriumammonium-phosphat	14	19,0	51,0	32,5	23,3	254,3	6,50
6	Ammoniumacetat	12	18,0	49,0	34,5	25,3	274,6	9,73
7	Ammoniumlactat	10	17,5	52,0	29,5	20,3	219,5	6,15
8	Calciumchlorid	10	21,0	54,0	32,5	23,3	251,0	7,76
9	Acidum hydrochloricum	10	16,0	48,0	28,5	19,3	210,8	6,66
10	Acidum lacticum	10	22,5	43,0	29,5	20,3	219,5	5,27
11	Acidum aceticum	12	20,0	36,0	28,6	19,3	209,7	12,12
12	Natriumdihydrophosphat	10	24,0	50,0	36,5	27,3	294,5	10,72
13	Ammoniumhydrophosphat	10	16,5	43,0	30,5	21,3	234,8	7,10

1) Number of animals
2) Weight of the parathyroid in mg
3) minimal 4) maximal 5) average
6) weight increase of the adrenal glands, average value

3. Ammonium-sulfate was administered to 10 female rabbits during 5 - 16 months with the same method and in identical doses. When killed, their body weight was 4100 to 4800 g. The weight of the parathyroid was 15 to 33 mg, and 27,5 on the average, i.e. 18,3% more than the weight for the controls, which means a plus of 98,9%. Differentia significans: 7,04.

4. Ammonium-carbonate was used for treating 14 female rabbits during 5 to . . . (illeg.) months. Same treatment method. Body weight at the time when killed: 4000 to 4700 g. Weight of the parathyroid: 15 to 40 mg, and 25 mg on the average, i.e. 15,8 mg more than for the controls. Accordingly, the weight increase was 171,9%, with a significance of 6,86.

5. Sodium-ammonium-phosphate was likewise applied to 14 female rabbits during 5 to 14 months, with the same method. Body weight at the time when killed: 3800 to 4700 g. Weight of the parathyroid: 19 to 51 mg and 32,5 mg on the average, i.e. 23,3 mg more than for the untreated control animals. This is a weight increase with a significant difference of 6,5.

6. Ammonium-acetate was used for treating 12 female rabbits during 5 to 16 months. Identical treatment method. Body weight at the time when killed: 3500 to 4800 g. Weight of the parathyroid: 18 to 49 mg, average value: 34,5 mg, i.e. 25,3 more than for normal animals, which means a weight plus of 274,6%; significance: 9,73.

7. Ammonium lactate was used for the treatment of 10 female rabbits during 5 - 16 months. Same treatment method. Body weight at the time when killed: 3350 to 1700 g. Weight of the parathyroid : 17,5 to 52 mg and 29,5 on the average, i.e. 20,3 mg more than for the controls, which means a weight increase of 219,5%. Significance: 6,15.

8. Calcium-chloride was used for treating 10 female rabbits during 5 - 16 months with the same method. Body weight when killed: 3700 to 5050 g, weight of the parathyroid 21 to 54 mg, 32,5 mg on the average, i.e. 23,3 mg more (251%) than for the controls. Differentia significans: 7,76.

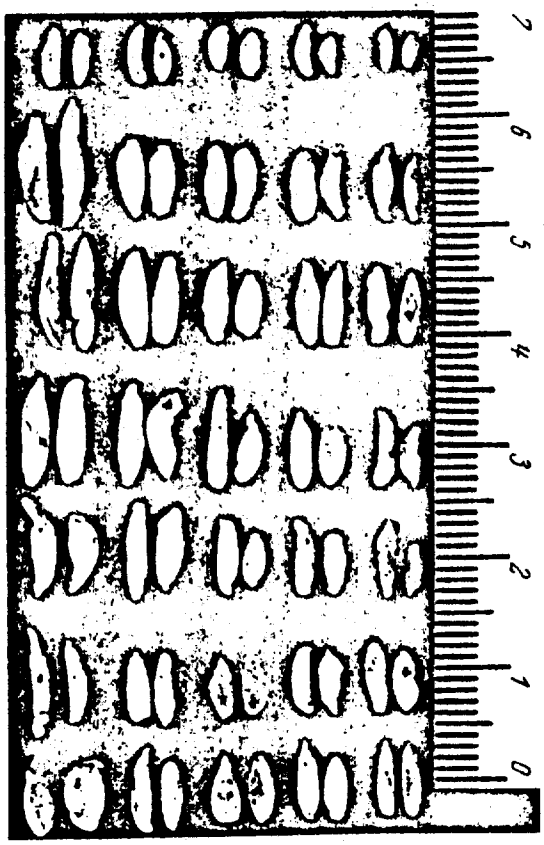
9. Acidum hydrochloricum was used for the treatment of 10 female rabbits during 5 to 16 months. The animals received twice daily 0,5 to . . . (illeg.) 25% HCL in 100 to 150 cm^3 of drinking water, in gradually increasing doses. A 3-week treatment period was also followed here by a pause of one week without treatment. Body weight when killed: 3500 to 4500 g. Weight of the parathyroid 16 to 48 mg, and 28,5 mg on the average, i.e. 19,3 mg more than for normal animals. This means a weight plus of 210%, with a significance of . . . (illegible).

10. Acidum lacticum was applied to 10 female rabbits during 5 to 16 months. The animals received increasing doses of 0,2 -0,5 cm^3 active substance twice daily in 100 to 150 cm^3 of drinking water. A 3-week treatment period was followed by a pause of one week. When killed, the body weight was 3700 to 4800 g.--Weight of the parathyroid 22,5 to 43 mg, and 29,5 mg on the average, i.e. 20,3 mg (219,5%) more than for the controls. Significant difference: 5.27.

11. Acidum aceticum was used for the treatment of 12 female rabbits during 5 to 16 months. The animals received every second day increasing doses of . . . (illeg.) 0,5 cm^3/kg concentrated acetic acid in 100 to 150 cm^3 of drinking water. Body weight at the time when killed: 3600 to 4600 g. Weight of the parathyroid 20 to 36 mg, average value 28,6 mg, i.e.19,3 mg more than for the normal rabbits, which corresponds to an increase of 209,7%. Differentia significans: 12,12.

12. Sodium hydrophosphate was used for the treatment of 10 female rabbits during 5 to 16 months. These animals received, however, 0,3 to 0,7 g/kg of the active substance twice daily in increasing doses, in 100 to 150 cm^3 of drinking water. Body weight when killed: 4000 to 4500 g. Weight of the parathyroid 24 to 50 mg, average value: 36,5 mg, i.e. 27,3 mg more than for the controls. This means a 294,5% weight increase, with a significance of 10,72.

13. <u>Ammonium-hydrophosphate</u> was administered to 10 female rabbits for 5 to 16 months. Each animal received 0,3 to 0,7 g/kg of the compound twice daily in increasing doses, in 100 to 150 cm^3 of drinking water. Body weight at the time when killed: 3900 to 4800 g. Weight of the parathyroid 16,5 to 43 mg, average value: 30,5 mg, i.e. 21,3 mg (234,8%) more than for the controls. Significant difference: 7,1.



<u>Illustration 1</u>. Enlargement of the parathyroid in rabbits treated with various compounds during 5 months.
Line I: ammonium-chloride treatment: 16,5 - 18 - 21,5 - 57,5 mg.--Line II: ammonium-sulfate treatment: 15 - 17 - 20 - 27,5 mg.--Line III: ammonium carbonate treatment: 15 -18 - 20 - 27,5 to 40 mg.-- Line IV: Na-ammoniumphosphate treatment: 19 - 20,5 -37,5 - 51 mg. Line V: ammonium acetate treatment: 18 - 22.5 - 24 - 39 mg. Line VI: ammonium lactate treatment: 17,5 - 20 - 21 - 30 - 52 mg. Line VII: controls without treatment: 6 - 7 - 8 to 9,5 - 10 mg.

It can be stated in view of the cited data that under the effect of the applied compounds the weight of the parathyroid increases considerably, i.e (its increase) is maximal with. . .(illeg.)sodiumhydrophospahte (294,5%), ammonium-chloride and ammonium-acetate (274,6%) respectively; it is minimal in animals treated with ammonium-carbonate (171,9%0 and ammonium-hydroxide (182,6%). On the basis of the statistical computation results the weight increase of the adrenal glands can unanimously be qualified as specific. --The weight increase coincided with a conspicuous enlargement of the adrenal gland volume and dimensions respectively.

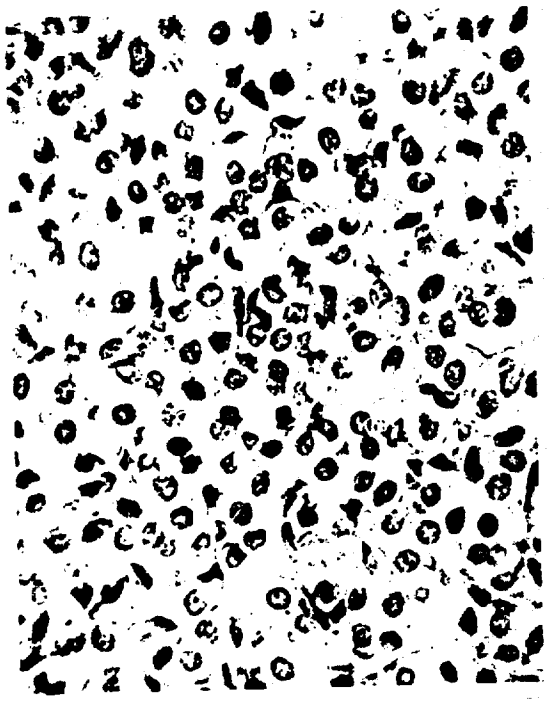


Illustration 2. Histological aspect of the parathyroid in untreated control rabbits. Dark primary cells of the gland substance predominate. The . . .(illeg.) primary cell number is comparatively small; their size is normal. Hematoxyline-eosin pigment. 1000-fold enlargement.

It was found in the course of the histological examination that the parathyroid vessels of the untreated controls were generally moderately plethoric and moderately wide. The main mass of the glands consisted of the so-called dark primary cells; oxyphilic cells could be found at gland edge sectors (Ill.2). -- In the treated rabbits, on the other hand, the larger vessels, but also the capillaries were wide and filled with blood already after a 3-month treatment period; the main mass of the glands consisted of enlarged and also numerically increased so-called light primary cells. The plasma of these cell types was enlarged, light and it contained vacuoles. Their nucleus was likewise large and light when colored . Nuclear division could be observed in several of these cells. Only a comparatively small number of dark primary cells was detected in the parathyroid of the treated animals.

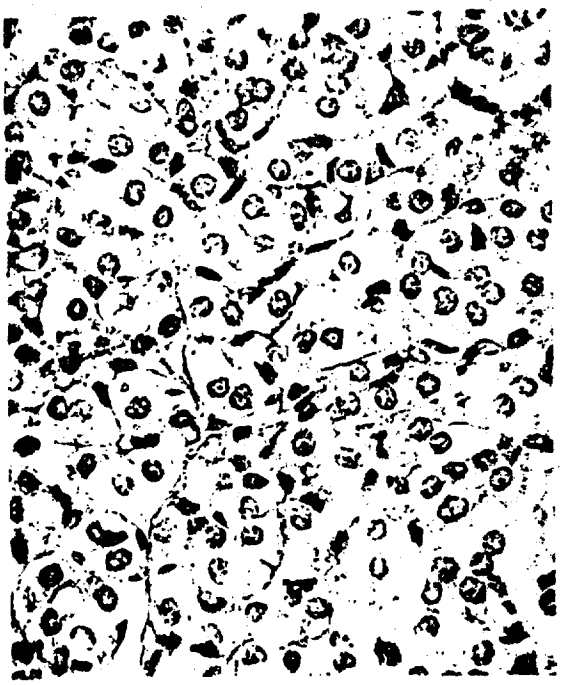


Illustration 3. Histological aspect of the enlarged parathyroid of a rabbit treated during 3 months with NH_4Cl. The gland substance is principally formed by light primary cells with enlarged plasma. Hemotoxyline-eosin coloring. 1000-fold enlargement.

A similar histological aspect was found also by ANSELMINO, HOFFMANN and HEROLD in rats, by HERTZ and KRANES in rabbits, by HOUSSAY in dogs and rabbits after the application of a watery HVL-extract. The authors are of opinion that this symptom is a consequence of the stimulating effect of HVL on the function of the adrenal glands. According to HOUSSAY's findings atrophy of the cell protoplasm, decrease of the cells number and indistinct cell limits were observable already 5 days after hypophysectomy. The regular epithelial structure of the glands disappeared simultaneously, and a trabecular, bundle-like cell system developed. Said atrophy of the parathyroid was especiall conspicuous in dogs which had undergone simultaneous surgical removal of the hypophysis and of the pancreas. VERNETTI observed a considerable increase of the connective tissues in atrophied parathyroid of dogs after hypophysectomy.

In the course of my own tests , plethora, hypertrophy and hyperplasia, a considerable multiplication of the light primary cells and symptoms of nuclear division were evident in the adrenal glands of treated rabbits. On the strength of all this, the conclusion could be reached that an intensified function of the parathyroid occurs, caused by the treatment which was used. However, we will be able to state a definite oponion on the above only after subjecting the enlarged adrenal glands to function tests.

The question now arises: what constitutes the function mechanism of the observed enlargement of the parathyroid caused by treatment? Since the 13 applied compounds include organic and inorganic, ammoniacal and non-ammoniacal compounds as well as acids and lye solutions, it is unconceivable that the described adrenal gland enlargement could result from the specific effect of said compounds. Efforts must therefore be made to detect a common cause related to the action mechanism, which always occurs in the organism under the effect of said compounds.

It is generally known that the acids and acid . . . (illegible word) shift the acid-base equilibrium of the organism towards acidity (acidosis); it is less well-known, however, that the lye solutions can cause acidosis as well. FAZEKAS demonstrated that NH_4OH and NaOH--despite their lye characteristics--cause acidosis in the organism. His statements were also confirmed by VENULET, GOEBEL and TISLOWITZ, ALVALI and GEIGER , as well as by HAZARD and VAILLE.The shared characteristic of the used compounds therefore consists in their ability to shift the acid-base equilibrium of the organism in the acidotic direction. We believe that the shift of the blood chemistry towards acidity has an irritating effect, either directly on the HVL, or by way of the hypothalamus center of the hypophysis, affecting the corresponding cells of the HVL, thereby stimulating them into intensified functioning.

Then the intensified hypophysis function causes the enlargement and intensified function respectively, of the parathyroid Our findings to the effect that the hypophysis of such treated animals is likewise enlarged and that the basophil cell number of the HVL increases, speaks in favor of the above. Our previous statements according to which the effect of these compounds in rabbits causes enlargement and intensified functioning of the suprarenal gland core, follicle maturing and (follicle) hemorrhage in the ovaries as well as the formation of yellow corpuscles, also speak in favor of an increased HVL-function.

The following data in literature refer to an intensified function of the hypophysis under acidoctic effect: JULESZ found follicle maturation in infantile mice ovaries after injecting male and female urine and with ketogenic diet. SELYE and HERLANT observed similar symptoms, besides hylerplasia of the suprarenal gland core after injecting hydrochloric acid intravenously. HARRIS injected a cuprunacetate solution into the 3. cerebral chamber of rabbits, which triggered ovulation by irritating the hypothalamus center. A chronic acidosis is determinable mostly for those diseases of man during which, according to data in litarature, an enlargement of the adrenal glands was found; for example: in cases of chronic kidney ailments, pregnancy, partial or total kidney extirpation, etc.

After the administration of sodium- and ammonium-phosphate to rabbits TORNBLOM observed enlargement of the adrenal glands, simultaneous decrease of the blood calcium and a considerable increase of phosphorus in the blood. He repeated the treatment, using animals whose hypophysis had been surgically removed; he subsequently found a decrease of the blood calcium- and phosphorus content, but no parathyroid enlargement took place. TORNBLUM concluded on the basis of the above that the enlargement of the adrenal gland is caused by hyperphosphatemia. He used other test animals to investigate which hormone effect is correlated to the modification of the phosphoremia. He removed the parathyroid and the thyroid glands of one animal group, and removed the thyroid glands, parathyroid and hypophyses of another group. The hypocalemia and hyperphospatemia of these animals was less extensive. However, when the animals without thyroid- and adrenal glands received hypophysis extract, the hylocalcemia did not change, but the hyperphosphatemia was considerably higher. In his opinion, this indicates that the hypophysis intensifies the phosphoremia, without requiring the intervention of the adrenal glands. TORNBLUM demonstrates the effect of the hypophysis schematically as follows: hypophysis - hyperphosphatemia - hyperfunction of the adrenal gland. In his view, the adrenal gland is not directly irritated by the hypophysis; instead, this occurs through the humoral factor, by increasing the blood phosphorus level.

The result of our own tests seems to contradict TORNBLOM's opinion, since we applied--besides 3 types of phosphates--10 other phosphorus-free compounds and observed an enlargement of the parathyroid caused by them. It was stated in the course of previous investigations (FAZEKAS) that the inorganic blood phosphorus content of rabbits is considerably increased by a single administration of a larger dose of NH_4OH and NaOH, while the serum-calcium and serum-chlorine decreases, hyperglycemia occurs, the alkali reserve of the blood serum declines (!) and the concentration of the hydrogenion increases, i.e. acidosis is generated. Therefore hyperphosphatemia can likewise occur without phosphorus supply. According to our experiences, an increase of the inorganic blood phosphorus content in case of acidotic conditions due to various causes (intoxications, diseases) is almost always detectable. This fact seems to confirm TORNBLOM's interpretation. According to BALO's extensive and thorough investigations the inorganic phosphorus content of the blood serum increases considerably in case of a subsequent blood acidification in vitro as well., without being influenced by the hypophysis here. Accordingly, the phosphorus content of the blood serum can increase without hypophysis action (the organically bound P of the red corpuscles is transformed into an inorganic bond and enters the blood serum). All this indicates that the increase of the blood phosphorus content can be regarded as a partial symptom of the acidosis, and that its presence alone offers no incontestable evidence (to confirm the assumption) that the enlargement of the parathyroid and their intensified function respectively, are caused by hyperphosphatemia.

In our opinion--on the basis of our own and other test results - the enlargement and the increased function respectively, of the parathyroid occurs as follows: the shift of the blood chemistry towards acidity due to various causes has an irritating effect on HVL. either directly, or by way of the hypothalamus center of the hypophysis;,it then stimulates this organ, intensifying its function, which subsequently causes the enlargement of the adrenal glands through the formation of parathyrcotrophormone.

Present investigations offer new data to show that the inner-secretory gland system can be chemically influenced and its functtion changed respectively, without hormone application, through the administration of simple compounds. -- Deceive statements regarding the adrenal gland, however, can be made on the basis of new results only. Pertinent investigations are in course and it is hoped that the hypofunction of the parathyroid as well as pathologic conditions caused by the same can likewise be remedied.

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SUMMARY

1. 362 rabbits were given twice daily for 3-5-16 months organic and inorganic ammonium-and other compounds (0.1 - 0.2 gl kg). These compounds had the same quality in that they shifted the acid-base equilibrium of the organisms in the acid direction. The weight, size and histological picture of the parathyroids of the treated rabbits were compared with the corresponding information about 50 untreated control animals of the same age, species and weight.
2. The minimum weight of the parathyroid in the controls amounted to 6 mg, the maximum 14 mg, and the acerage value 9.2 mg.
3. In contrast, the minimum weight of the parathyroids of the acid-treated rabbits amounted to 15-25 mg, the maximum 33-57.5 mg, and the average value 25-36.5 mg. The average weight showed, in proportion to the control animals, an increase of 15.8-27 mg, that is 171.9-294.5%, which clearly signified statistically a significant difference of 5.27-12.12.
4. Histologically, the substance of the parathyroids of the control rabbits consisted mainly of dark primary cells; light primary cells existed in a comparatively small number; oxyphilic cells were scattered in the edge sectors. In the enlarged parathyroids of the treated animals a wealth of blood was ascertained, the dark primary cells decreased in number considerably; the oxphilic cells were missing or likewise decreased in number; the light primary cells were considerably increased and enlarged, in several places, with vacuole formation, such that the primary mass of the gland consisted of the light primary cells.

5. By reason of the enlargement and of the histological pictures of the parathyroids, it was concluded that their function increased. However, we will be able to state a definite opinion on the above only after subjecting the enlarged parathyroids to function tests.

6. Our earlier assertions - that enlargement of the hypophysis, increase of the basophilic cells of the HVL, enlargement of the adrenal cortex and intensification of its functions, enlargement of the ovaries, follicle maturity and bleeding, corpus lutem formation, enlargement of the uterus and milk secretion can be produced in animals treated such - indicated that these changes as well as the enlargement of the parathyroids are connected with the increased function of the HVL.

7. The increased function of the HVL, in our opinion, did not result from the specific effect of the applied compounds, but rather resulted from the moderates, periodic, acidic influence caused by them.

8. Our investigations therefore offer a new proof that the function of the inner-secretory gland-system can be transposed or increased through administration of simple compounds with "acid effects". By reason of our results it is hoped that the function of the hypo-functioning parathyroids (and other inner-secretory glands) can be restored or made normal and at the same time the pathological conditions caused by this hypofunction can be remedied.

Fazekas-'54

Bib No. 114

Virchows Archiv, Bd. 324, S. 531—542 (1954).

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(Direktor: Prof. Dr. I. Gy. Fazekas).

Vergrößerung der Nebenschilddrüsen durch einfache acidotische Verbindungen.

Von

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Mit 3 Textabbildungen.

(Eingegangen am 7. Juli 1953.)

Über Vergrößerung menschlicher Nebenschilddrüsen wurde zuerst von Erdheim (1903), später von Claude und Baudouin, Reinhard und Creuzfeld, Ballet und Lavastine, Josephson, Erdheim, Cushing und Davidoff, Hoff u. a. in Verbindung mit basophilen Adenomen des Hypophysenvorderlappens (HVL) sowie von Lloyd in Verbindung mit Hauptzellenadenomen der Hypophyse berichtet. Recklinghausen beschrieb 1904 das Krankheitsbild der Osteitis fibrosa generalisata, die sich unter dem Einfluß der adenomisch oder hyperplastisch bedingten gesteigerten Nebenschilddrüsenfunktion ausbildet. Der Zusammenhang zwischen dieser Knochen- und Nebenschilddrüsenveränderung bzw. -hyperfunktion wurde zuerst von Mandl (1926) und später von anderen dadurch bewiesen, daß die Kranken nach operativer Entfernung ihrer adenomischen oder hyperplastischen Nebenschilddrüsen gesund wurden. Die sog. *sekundäre Hyperplasie* der Nebenschilddrüsen kann als Kompensationsreaktion bei gesteigertem Parathormonbedürfnis des Organismus vorkommen, so z. B. bei Knochenmetastasen eines Carcinoms, bei einem Myeloma multiplex, einer Rachitis, Osteomalacie, Cushingschen Krankheit, akuten Nierenerkrankung (Cameron, Verzár), bei Gravidität (Árvay), bei partieller oder totaler Nierenexstirpation sowie bei einem Calcium- und Phosphorgehaltzuwuchs der Speiseordnung (Cournot).

Angelmino, Hoffmann und Herold beobachteten nach mehrtägiger Verabreichung von HVL-Extrakt an Ratten, später auch an anderen Tieren, Hertz und Hanes an Kaninchen, Ham und Hais an Hunden Vergrößerungen der Nebenschilddrüse. Auf Grund dieser Beobachtungen nahmen sie an, daß der HVL ein die Nebenschilddrüsenfunktion stimulierendes Hormon bildet. Damit stehen in vollem Einklang die Beobachtungen, nach denen Smith an Ratten, Köster, Houssay und Biasotti, sowie Houssay und Sammartino, aber auch Vernetti an Hunden nach Exstirpation der Hypophyse eine Atrophie und degenerative Atrophie der Nebenschilddrüsen vorfinden konnten. Livon und Peyron sowie Aschner sahen hingegen an Hunden, später Collip an Ratten keine Schilddrüsenatrophie nach Entfernung der Hypophyse. Thomison und Cushing konnten an Hunden, Cattaneo an Kaninchen durch Hypophysenextrakt keine Vergrößerung der

Nebenschilddrüsen erzielen. 1949 beobachtete TORNHOM an Kaninchen nach Verabreichung von großer Menge Natrium- und Ammoniumphosphat bei einer 12wöchigen calciumarmen Speisefolge eine hochgradige Vergrößerung der Nebenschilddrüse.

Die bisherigen Untersuchungen stellten klar, daß die gesteigerte oder verminderte Funktion der Nebenschilddrüsen durch die im Calcium- und Phosphorstoffwechsel des Organismus verursachten tiefgreifenden Veränderungen verschiedene pathologische Zustände hervorruft. Es wäre also wünschenswert, die Funktion der Nebenschilddrüse auf chemischem Wege so beeinflussen zu können, daß die durch ihre verminderte Funktion bedingten Erkrankungen durch die Wiederherstellung der Funktion zu beheben wären.

Im Laufe früherer Untersuchungen (1938—1950) wurde schon festgestellt (FAZEKAS), daß durch dauerhafte Verabreichung zahlreicher acidotisch wirkender Verbindungen in Kaninchen, Gänsen, Schweinen und Ziegen Hypertrophie und gesteigerte Funktion der Nebennierenrinde, Vergrößerung der Ovarien und des Uterus, Follikelreifung und -blutung, Gelbkörperbildung, cyclische Veränderungen an der Schleimhaut der Uterushörner, gesteigertes Fettwerden, Vergrößerung der Brüste und Milchsekretion sowie Vergrößerung der Hypophyse und Vermehrung der basophilen Zellen des HVL erzielt werden konnten. — Auf die Möglichkeit der chemischen Beeinflussung der Nebenschilddrüsen sollen folgende Untersuchungen hinweisen.

Zur Behandlung der Tiere wurden die in der Tabelle angeführten Verbindungen verwendet. Die Nebenschilddrüsen von 362 behandelten Kaninchen (darunter befanden sich auch Tiere aus vorhergehenden Versuchen) wurden untersucht, Größe, Gewicht und histologischer Befund mit den entsprechenden Angaben der Kontrolltiere verglichen. Als Kontrollen dienten 50 gleichaltrige und -artige Kaninchen mit gleichem Gewicht. — Aus der Übersichtstabelle geht es hervor, daß die Nebenschilddrüsen der unbehandelten Kontrolltiere 6—14 mg, im Mittelwert 9,2 mg wogen, die der behandelten Kaninchen hingegen an Gewicht erheblich zunahmen.

1. Mit *Ammoniumhydroxid* wurden 96 weibliche und 64 männliche Tiere 4—5—16 Monate lang behandelt. Jedes Tier bekam 2täglich 50 bis 80 cm³ $^1/_2$%iges NH_4OH durch Magensonde in allmählich zunehmenden Dosen. Nach einer 3wöchigen Behandlung folgte immer eine 1wöchige behandlungsfreie Periode. Das Körpergewicht der Tiere nahm im Laufe der Behandlung allmählich zu und betrug bei der Tötung mit Luftembolie 3100—4400 g. — Die Nebenschilddrüsen wogen 17—39 mg, im Mittelwert 26 mg, was im Verhältnis zum Gewicht der Nebenschilddrüsen der Kontrollen um 16,8 mg, d. h. um 182,6% mehr war. Auf

Tabelle 1. *Gewichtsangaben der Nebenschilddrüsen bei behandelten Kaninchen und Kontrollen.*

		Zahl der Tiere	Gewicht der Nebenschilddrüsen in mg			Gewichtszunahme der Nebenschilddrüsen im Mittelwert		S. D.
			minimal	maximal	Mittelwert	%	mg	
	Kontrollen	50	6	14	9,2			
1	Ammoniumhydroxyd	160	17,0	39,0	26,0	16,8	182,6	10,84
2	Ammoniumchlorid	80	16,5	57,5	34,5	25,3	274,6	9,03
3	Ammoniumsulfat	10	15,0	33,0	27,5	18,3	198,9	7,04
4	Ammoniumcarbonat	14	15,0	40,0	25,0	15,8	171,9	6,86
5	Natriumammoniumphosphat	14	19,0	51,0	32,5	23,3	254,3	6,50
6	Ammoniumacetat	12	18,0	49,0	34,5	25,3	274,6	9,73
7	Ammoniumlactat	10	17,5	52,0	29,5	20,3	219,5	6,15
8	Calciumchlorid	10	21,0	54,0	32,5	23,3	251,0	7,76
9	Acidum hydrochloricum	10	16,0	48,0	28,5	19,3	210,8	6,66
10	Acidum lacticum	10	22,5	43,0	29,5	20,3	219,5	5,27
11	Acidum aceticum	12	20,0	36,0	28,6	19,3	209,7	12,12
12	Natriumdihydrophosphat	10	24,0	50,0	36,5	27,3	294,5	10,72
13	Ammoniumhydrophosphat	10	16,5	43,0	30,5	21,3	234,8	7,10

Grund der statistischen Rechnungen betrug die signifikante Differenz 10,84, die Gewichtszunahme der Nebenschilddrüsen ist also spezifisch.

2. Mit *Ammoniumchlorid* wurden 50 weibliche und 30 männliche Kaninchen 3—5—16 Monate behandelt. Zweitäglich bekamen die Tiere 0,1—0,2 g/kg NH_4Cl in 100—150 cm³ Trinkwasser gelöst, in allmählich zunehmenden Dosen. Nach 3wöchiger Behandlung wurde immer eine Behandlungspause von 1 Woche eingeschaltet. Das Gewicht der Tiere nahm während der Behandlungsdauer allmählich zu und betrug bei der Tötung mit Luftembolie 3500—4500 g. Das Gewicht der Nebenschilddrüsen betrug 16,5—57,5 mg, im Mittelwert 34,5 mg, das ist um 25,3 mg mehr als bei den Kontrollen, das einer 274,6%igen Gewichtszunahme entspricht. Die Signifikanz wurde für 9,03 gefunden.

3. *Ammoniumsulfat* wurde 10 weiblichen Kaninchen 5—16 Monate auf gleiche Art und in gleichen Dosen verabreicht. Körpergewicht bei Tötung 4100—4800 g. Gewicht der Nebenschilddrüsen 15—33 mg, im Mittelwert 27,5 mg, um 18,3 mg mehr als bei den Kontrollen, was 198,9%iges Plus bedeutet. Differentia significans: 7,04.

4. Mit *Ammoniumcarbonat* wurden 14 weibliche Kaninchen 5 bis 16 Monate behandelt. Gleiche Behandlungsmethode. — Körpergewicht bei Tötung: 4000—4700 g. Gewicht der Nebenschilddrüsen 15—40 mg,

im Mittelwert 25 mg, um 15,8 mg mehr, als bei den Kontrollen. Die Gewichtszunahme betrug also 171,9% mit einer Signifikanz von 6,86.

5. *Natriumammoniumphosphat* wurde gleichfalls 14 weiblichen Kaninchen 5—14 Monate mit gleicher Methode gegeben. Körpergewicht bei Tötung: 3800—4700 g. Gewicht der Nebenschilddrüsen 19—51 mg, im Mittelwert 32,5 mg, d. h. um 23,3 mg mehr als bei den unbehandelten Kontrolltieren. Das ist eine 254,3%ige Gewichtszunahme mit einer signifikanten Differenz von 6,5.

6. *Ammoniumacetat* wurde 12 weiblichen Kaninchen 5—16 Monate lang verabreicht. Behandlungsmethode wie vorher. Körpergewicht bei Tötung: 3500—4800 g. Die Nebenschilddrüsen wogen 18—49 mg, im Mittelwert 34,5 mg, um 25,3 mg mehr als bei den normalen Tieren; ein Gewichtsplus von 274,6%. Differentia significans 9,73.

7. Mit *Ammoniumlactat* wurden 10 weibliche Kaninchen während 5—16 Monate behandelt. Gleiche Behandlungsmethode. Körpergewicht bei Tötung: 3350—4700 g. Gewicht der Nebenschilddrüsen 17,5 bis 52 mg, im Mittelwert 29,5 mg, das ist um 20,3 mg mehr als bei den Kontrollen, das eine 219,5%ige Gewichtszunahme bedeutet. Signifikanz: 6,15.

8. Mit *Calciumchlorid* wurden 10 weibliche Kaninchen 5—16 Monate gleicherweise behandelt. Körpergewicht bei Tötung: 3700—5050 g. Gewicht der Nebenschilddrüsen 21—54 mg, im Mittelwert 32,5 mg, d. h. um 23,3 mg (251%) mehr als bei den Kontrollen. Differentia significans: 7,76.

9. Mit *Acidum hydrochloricum* wurden 10 weibliche Kaninchen 5 bis 16 Monate lang behandelt. Diese Tiere bekamen 2täglich 0,5—1,0 cm³ 25%iges HCl in 100—150 cm³ Trinkwasser, in allmählich zunehmenden Dosen. — Auch hier folgte nach einer Behandlung von 3 Wochen eine 1wöchige behandlungsfreie Pause. Körpergewicht bei Tötung der Tiere: 3500—4500 g. Gewicht der Nebenschilddrüsen 16—48 mg, im Mittelwert 28,5 mg, um 19,3 mg mehr als bei den normalen Tieren. Das bedeutet ein Gewichtsplus von 210,8%, mit einer Signifikanz von 6,66.

10. *Acidum lacticum* wurde 10 weiblichen Kaninchen 5—16 Monate hindurch gegeben. Den Tieren wurde 2täglich in zunehmenden Dosen 0,2—0,5 cm³/kg Wirkungsstoff in 100—150 cm³ Trinkwasser verabreicht. Nach 3 Wochen Behandlung folgte 1 Woche Pause. Bei Tötung betrug das Körpergewicht 3700—4800 g. — Gewicht der Nebenschilddrüsen 22,5—43 mg, im Mittelwert 29,5 mg, also um 20,3 mg, d. h. um 219,5% mehr als bei den Kontrollen. Signifikante Differenz 5,27.

11. Mit *Acidum aceticum* wurden 12 weibliche Kaninchen 5—16 Monate behandelt. Die Tiere bekamen 2tägig in zunehmenden Dosen 0,2 bis 0,5 cm³/kg konzentrierte Essigsäure in 100—150 cm³ Trinkwasser. Körpergewicht bei Tötung 3600—4600 g. Gewicht der Nebenschild-

drüsen 20—36 mg, im Mittelwert 28,6 mg, d. h. um 19,3 mg mehr als bei den normalen Kontrolltieren, was einer Zunahme von 209,7% entspricht. Differentia significans 12,12.

12. Mit *Natriumdihydrophosphat* wurden 10 weibliche Kaninchen 5—16 Monate lang behandelt. Diese Tiere bekamen aber 0,3 bis 0,7 g/kg Wirkungsstoff 2täglich in zunehmenden Dosen in 100 bis 150 cm³ Trinkwasser. Körpergewicht bei Tötung: 4000—4500 g. Gewicht der Nebenschilddrüsen betrug 24—50 mg, im Mittelwert 36,5 mg, also um 27,3 mg mehr als bei den Kontrollen. Das bedeutet eine 294,5%ige Gewichtszunahme mit einer Signifikanz von 10,72.

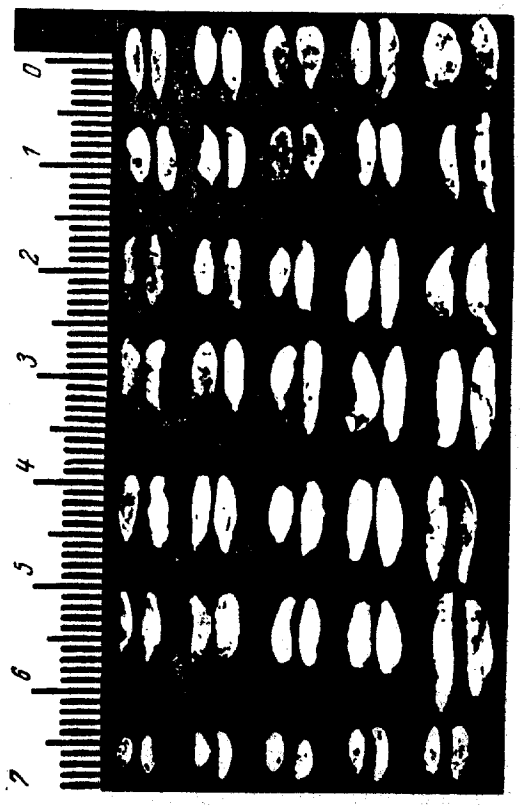

Abb. 1. Vergrößerung der Nebenschilddrüsen bei 5 Monate lang mit verschiedenen Verbindungen behandelten Kaninchen. — I. Reihe: Ammoniumchloridbehandlung: 16,5—18—24,5—26—57,5 mg. — II. Reihe: Ammoniumsulfatbehandlung: 15—16—17—20—27,5 mg. — III. Reihe: Ammoniumcarbonatbehandlung: 15—18—20—27,5—bis 40 mg. IV. Reihe: Na-Ammoniumphosphatbehandlung: 19—20,5—25—37,5—51 mg. V. Reihe: Ammoniumacetatbehandlung: 18—22,5—24—39—43 mg. VI. Reihe: Ammoniumlactatbehandlung: 17,5—20—24—30—52 mg. VII. Reihe: Kontrollen ohne Behandlung: 6—7—8 bis 9,5—10 mg.

13. *Ammoniumhydrophosphat* wurden 10 weiblichen Kaninchen 5 bis 16 Monate hindurch verabreicht. Ein Tier bekam 0,3 bis 0,7 g/kg Verbindung 2täglich in zunehmenden Dosen in 100 bis 150 cm³ Trinkwasser. Körpergewicht bei Tötung: 3900—4800 g. Gewicht der Nebenschilddrüsen 16,5—43 mg, im Mittelwert 30,5 mg, d. h. um 21,3 mg mehr (234,8%) als bei den Kontrollen. Signifikante Differenz: 7,1.

Mit Hinsicht auf die angeführten Angaben kann behauptet werden, daß die Nebenschilddrüsen unter Wirkung der verwendeten Verbindungen

an Gewicht erheblich zunehmen, und zwar maximal bei den [illegible] Natriumdihydrophosphat (294,5%), Ammoniumchlorid bzw. Ammoniumacetat (274,6%), minimal bei den mit Ammoniumcarb[illegible] (171,9%) und Ammoniumhydroxid (182,6%) behandelten Tie[illegible] Auf Grund der statistischen Rechnungsergebnisse kann die Gewi[illegible] zunahme der Nebenschilddrüsen einstimmig für spezifisch [illegible]

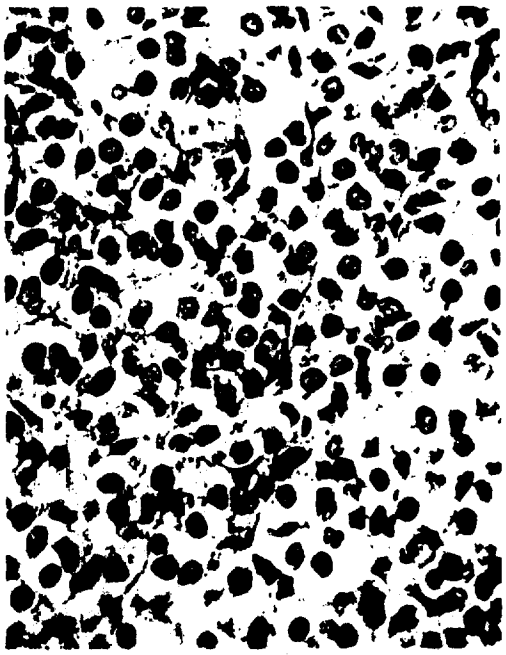

Abb. 2. Histologisches Bild der Nebenschilddrüsen bei unbehandelten Kontrollka[illegible] In überwiegender Mehrheit bilden dunkle Hauptzellen die Drüsensubstanz. Die h[illegible] Hauptzellen sind in verhältnismäßig kleiner Zahl und von normaler Größe. Hämatoxylin-Eosinfärbung. 1000fache Vergrößerung.

werden. — Die Gewichtszunahme ging mit einer auffallenden Vergrößerung der Nebenschilddrüsen an Volumen bzw. Dimensionen einher (Abb. 1).

Im Laufe der *histologischen Untersuchung* wurde beobachtet, da die Gefäße der Nebenschilddrüsen bei den *unbehandelten* Kontrollen im allgemeinen mittelmäßig blutreich und mittelmäßig weit waren. Die Hauptmasse der Drüsen bildeten die sog. *dunklen Hauptzellen*, an den Drüsenrandteilen konnten zerstreut *oxyphile Zellen* vorgefunden werden (Abb. 2). — In den Nebenschilddrüsen der *behandelten* Kaninchen hingegen waren die größeren Gefäße, aber auch die Capillaren, schon nach 3monatiger Behandlung weit und blutvoll, die Hauptmasse der

Drüsen bestand aus vergrößerten und auch an Zahl vermehrten sog. *hellen Hauptzellen*. Das Plasma dieser Zelltypen war vergrößert, hell, oft befanden sich darin Vacuolen. Ihr Kern war gleichfalls groß und färbte sich hell. In mehreren solchen Zellen waren Kernteilungen zu sehen. Dunkle Hauptzellen wurden in den Nebenschilddrüsen der behandelten Tiere nur in verhältnismäßig geringer Zahl vorgefunden.

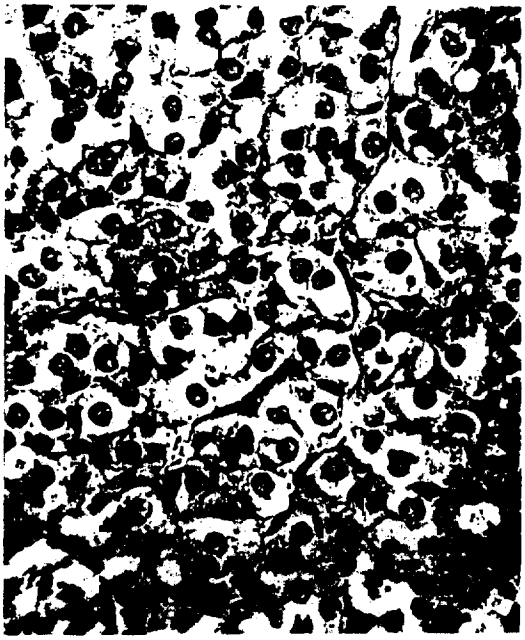

Abb. 3. Histologisches Bild der vergrößerten Nebenschilddrüsen eines 3 Monate lang mit NH_4Cl behandelten Kaninchens. Die Drüsensubstanz ist besonders von hellen Hauptzellen mit vergrößertem Plasma gebildet. — Hämatoxylin-Eosinfärbung. 1000fache Vergrößerung.

Die oxyphilen Zellen fehlten fast vollkommen in den 3—5monatigen Fällen, in den Fällen von 16 Monaten waren sie aber schon meistens aufzufinden. Vermehrung des Bindegewebes konnte nicht einmal nach 16monatiger Behandlung in den Nebenschilddrüsen der Tiere beobachtet werden (Abb. 3).

Gleiches histologisches Bild fanden auch ANSELMINO, HOFFMANN und HEROLD bei Ratten, HERTZ und KRANES bei Kaninchen, HOUSSAY bei Hunden und Kaninchen nach Verabreichung wäßrigen HVL-Extraktes. Verfasser betrachten diese Erscheinungen als Folge der auf die Funktion der Nebenschilddrüsen ausgeübten stimulierenden HVL-Wirkung. Nach den Beobachtungen von HOUSSAY konnten schon 5 Tage

nach der Hypophysektomie Atrophie des Zellprotoplasmas, Verminderung der Zellen und Verschwommenheit der Zellgrenze festgestellt werden. Gleichzeitig verschwand die gleichmäßige epitheliale Struktur der Drüsen, und es bildete sich eine trabeculare, bundelartige Anordnung der Zellen aus. Solche Atrophie der Nebenschilddrüsen war besonders bei Hunden auffallend, an denen Hypophysektomie und Pankreasexstirpation gleichzeitig durchgeführt worden sind. VERNETTI beobachtete in den atrophischen Nebenschilddrüsen hypophysektomisierter Hunde auch erhebliche Vermehrung des Bindegewebes.

Im Laufe eigener Untersuchungen zeigten sich in den Nebenschilddrüsen der behandelten Kaninchen Blutreichtum, Hypertrophie und Hyperplasie, erhebliche Vermehrung der hellen Hauptzellen und Kernteilungserscheinungen. Auf Grund all dessen könnte auf die gesteigerte Funktion der infolge der verwendeten Behandlung vergrößerten Nebenschilddrüsen geschlossen werden. Darüber werden wir aber eine endgültige Meinung nur auf Grund der an den vergrößerten Nebenschilddrüsen durchgeführten Funktionsuntersuchungen äußern können.

Es ergibt sich nun die Frage, worin besteht der Funktionsmechanismus der beobachteten, behandlungsbedingten Vergrößerung der Nebenschilddrüsen. Da sich unter den 13 verabreichten Verbindungen organische und anorganische, ammoniakalische und nichtammoniakalische Verbindungen sowie Säuren und Laugen befinden, kann nicht daran gedacht werden, daß die beschriebene Vergrößerung der Nebenschilddrüsen durch die spezifische Einwirkung dieser Verbindungen bedingt werden könne. Es muß also, in betreff des Wirkungsmechanismus nach einer gemeinsamen Ursache gesucht werden, die im Organismus unter dem Einfluß dieser Verbindungen immer auftritt.

Es ist im allgemeinen bekannt, daß die Säuren und sauren Salze das Säurebasengleichgewicht des Organismus in die saure Richtung verschieben (Acidose), weniger bekannt ist es aber, daß auch die Laugen eine Acidose verursachen können. FAZEKAS wies nach, daß NH_4OH und NaOH — trotz ihrem Laugencharakter — im Organismus eine Acidose hervorrufen. Seine Behauptungen fanden auch von VENULET, GOEBEL und TISLOWITZ, ALVALL und GEIGER sowie von HAZARD und VAILLE Bestätigung. Die gemeinsame Eigenschaft der verwendeten Verbindungen besteht also darin, daß sie das Säurebasengleichgewicht des Organismus in die acidotische Richtung verschieben. Unserer Ansicht nach wirkt die Verschiebung des Blutchemismus in die Säurerichtung reizend, entweder unmittelbar auf den HVL oder auf dem Weg über das Hypothalamuszentrum der Hypophyse auf die entsprechenden Zellen des HVL und stimuliert sie dadurch zur gesteigerten Funktion. Die gesteigerte Hypophysenfunktion ruft dann die Vergrößerung bzw. die gesteigerte Funktion der Nebenschilddrüse hervor

Dafür spricht unser Befund, daß sich auch die Hypophyse bei solch behandelten Tieren vergrößert und die basophilen Zellen des HVL an Zahl zunehmen. Gleichfalls für die gesteigerte HVL-Funktion sprechen unsere früheren Behauptungen, daß durch die Einwirkung dieser Verbindungen bei Kaninchen Vergrößerung und gesteigerte Funktion der Nebennierenrinde, in den Eierstöcken Follikelreifung und -blutung sowie Gelbkörperbildung hervorgerufen werden.

Auf die Funktionssteigerung der Hypophyse unter acidotischem Einfluß weisen auch folgende Literaturangaben hin: JULESZ fand an infantilen Mäuseovarien Follikelreifung nach Einspritzung von Urin von Männern und Frauen an ketogener Diät. SELYE und HERLANT beobachteten, außer Hyperplasie der Nebennierenrinde, gleiche Erscheinungen nach intravenöser Einspritzung von Salzsäure. HARRIS spritzte, in die 3. Hirnkammer von Kaninchen, eine Cuprumacetatlösung, was durch die Reizung der Hypothalamuszentren eine Ovulation auslöste. Eine chronische Acidose ist meistens bei jenen menschlichen Erkrankungen nachzuweisen, wo laut den Literaturangaben eine Vergrößerung der Nebenschilddrüsen beobachtet wurde; z. B. bei chronischen Nierenerkrankungen, Schwangerschaft, partieller oder totaler Exstirpation der Nieren usw.

TÖRNBLOM beobachtete bei Kaninchen nach Verabreichung von Natrium- und Ammoniumphosphat Vergrößerung der Nebenschilddrüsen, gleichzeitige Abnahme des Blutcalciums und hochgradige Zunahme des Blutphosphors. Die Behandlung wurde von ihm an hypophysektomisierten Tieren wiederholt, danach eine Herabsetzung des Blutcalcium- und -Phosphorgehaltes festgestellt, die Nebenschilddrüsen vergrößerten sich aber nicht. TÖRNBLOM schloß daraus, daß die Vergrößerung der Nebenschilddrüse durch die Hyperphosphatämie verursacht wurde. In anderen Versuchsserien forschte er nach, von was für Hormoneinwirkungen die Veränderung der Phosphorämie abhänge. Er führte an einer Kaninchengruppe Schilddrüsen- und Nebenschilddrüsenentfernung, an der anderen Schilddrüsen-, Nebenschilddrüsen- und Hypophysenexstirpation durch. Bei diesen Tieren war die Hypocalcämie und Hyperphosphatämie kleiner. Wenn aber den schilddrüsen- und nebenschilddrüsenberaubten Tieren Hypophysenextrakt verabreicht wurde, veränderte sich die Hypocalcämie nicht, die Hyperphosphatämie war aber wesentlich höher. Das weist — seiner Meinung nach — darauf hin, daß die Hypophyse die Phosphorämie verstärkt, ohne die Vermittlung der Nebenschilddrüsen in Anspruch genommen zu haben. TÖRNBLOM veranschaulicht den Einfluß der Hypophyse auf die Nebenschilddrüse mit folgendem Schema: Hypophyse—Hyperphosphatämie—Hyperfunktion der Nebenschilddrüse. Nach seiner Auffassung geschieht die Reizung der Nebenschilddrüse nicht unmittelbar durch die Hypophyse, sondern auf dem Weg über einen humoralen Faktor durch die Steigerung des Blutphosphorniveaus.

Das Ergebnis unserer eigenen Versuche scheint dieser Ansicht von TÖRNBLOM zu widersprechen, da wir außer 3 Phosphatarten noch 10 andere phosphorfreie Verbindungen verabreicht haben und danach eine Vergrößerung der Nebenschilddrüse beobachten konnten. In vorigen Untersuchungen wurde behauptet (FAZEKAS), daß der anorganische Blutphosphorgehalt bei Kaninchen durch einmalige Verabreichung einer größeren Dosis von NH_4OH und NaOH erheblich anwächst, das Serumcalcium und -chlor abnimmt, eine Hyperglykämie auftritt, die Alkalireserve des Blutserums absinkt (!) und die Konzentration des Hydro-

genions zunimmt, d. h. eine Acidose hervorgerufen wird. Eine Hyperphosphatämie kann also auch ohne Phosphorzufuhr zustande kommen. Laut unseren Erfahrungen ist der Zuwachs des anorganischen Blut-Phosphor-Gehaltes bei den infolge verschiedenster Ursachen (Vergiftungen, Erkrankungen) auftretenden acidotischen Zuständen fast immer nachzuweisen. Diese Tatsache scheint TORNAI'S Auffassung zu unterstützen. Laut den ausgedehnten und gründlichen Untersuchungen von BALÓ nimmt der anorganische Phosphorgehalt des Blutserums auch im Falle des nachherigen Sauerwerdens des Blutes in vitro erheblich zu, ohne hier durch die Hypophyse beeinflußt zu sein. Demnach kann der Phosphorgehalt des Blutserums auch ohne Hypophyseneinwirkung ansteigen (das organisch gebundene P der roten Blutkörperchen geht in eine anorganische Bindung über und gelangt in das Blutserum), was das weist darauf hin, daß die Steigerung des Blutphosphorgehaltes Teilerscheinung der Acidose angesehen werden kann und ihr Dasein an sich keinen Beweis außer allem Zweifel dafür bietet, als ob die Vergrößerung der Nebenschilddrüsen bzw. ihre Funktionssteigerung durch die Hyperphosphatämie bedingt wäre.

Auf Grund eigener und anderer Versuchsergebnisse kommt die Vergrößerung bzw. gesteigerte Funktion der Nebenschilddrüse — unserer Ansicht nach — so zustande, daß die durch verschiedene Ursachen bedingte Verschiebung des Blutchemismus in die Säurerichtung entweder unmittelbar oder auf dem Weg über das Hypothalamuszentrum der Hypophyse auf den HVL reizend wirkt, dieses Organ zur gesteigerten Funktion stimuliert, was dann durch die gesteigerte Bildung von Parathyreotrophormon die Vergrößerung der Nebenschilddrüsen bzw. ihre gesteigerte Funktion verursacht.

Vorliegende Untersuchungen dienen mit neuen Angaben darüber, daß das innersekretorische Drüsensystem ohne Hormondarreichung bloß durch Zufuhr von einfachen Verbindungen auf chemischem Wege beeinflußt bzw. seine Funktion umgestellt werden kann. — Angesichts der Nebenschilddrüse kann aber Endgültiges nur auf Grund neuer Ergebnisse ausgesprochen werden. Die diesbezüglichen Untersuchungen sind im Gange und hoffentlich können Hypofunktion von Nebenschilddrüsen und auch dadurch bedingte pathologische Zustände behoben werden.

Zusammenfassung.

1. 362 Kaninchen wurden 2täglich 3—5—16 Monate hindurch organische und anorganische Ammoniak- und andere Verbindungen (0,1—0,2 g/kg) verabreicht. (Die gemeinsame Eigenschaft dieser Verbindungen besteht darin, daß sie das Säurebasengleichgewicht des Organismus in die Säurerichtung verschieben.) Das Gewicht, die Größe und das histo-

logische Bild der Nebenschilddrüsen solch behandelter Kaninchen wurde mit den entsprechenden Angaben von 50 unbehandelten gleichaltrigen Kontrollkaninchen von gleicher Art und gleichem Gewicht verglichen.

2. Das minimale Gewicht der Nebenschilddrüsen bei Kontrollen betrug 6 mg, das maximale 14 mg, im Mittelwert wurden 9,2 mg vorgefunden.

3. Im Gegensatz betrug das minimale Gewicht der Nebenschilddrüsen der acidotisch behandelten Kaninchen 15—25 mg, das maximale 33—57,5 mg, im Mittelwert also 25—36,5 mg. Diese Gewichtszunahme zeigt, im Verhältnis zu den Kontrolltieren, im Mittelwert 15,8—27 mg, d.h. 171,9—294,5%, auf, was laut statistischer Berechnungen eine signifikante Differenz von 5,27—12,12 bedeutet.

4. Histologisch bestand die Substanz der Nebenschilddrüsen bei den *Kontroll*kaninchen besonders aus *dunklen Hauptzellen*, helle Hauptzellen waren in verhältnismäßig kleiner Zahl vorhanden, an den Randzeilen lagerten auch oxyphile Zellen zerstreut. In den vergrößerten Nebenschilddrüsen der *behandelten Kaninchen* hingegen war Blutreichtum festzustellen, die dunklen Hauptzellen nahmen an Zahl erheblich ab, die oxyphilen Zellen fehlten sogar oder nahmen gleichfalls an Zahl ab, die *hellen Hauptzellen* vermehrten und vergrößerten sich aber erheblich, an mehreren Stellen mit Vacuolenbildung, so daß die Hauptmasse der Drüsen von den hellen Zellen gebildet wurde.

5. Auf Grund der Vergrößerung und des histologischen Bildes der Nebenschilddrüsen kann auf ihre gesteigerte Funktion geschlossen werden. Darüber werden wir uns aber nur nach der funktionalen Untersuchung der vergrößerten Nebenschilddrüsen aussprechen können.

6. Unsere früheren Behauptungen, daß bei so behandelten Tieren Hypophysenvergrößerung, Vermehrung der basophilen Zellen des HVL, Vergrößerung und gesteigerte Funktion der Nebennierenrinde, Vergrößerung der Eierstöcke, Follikelreifung und -blutung, Corpus luteum-Bildung, Uterusvergrößerung und Milchsekretion hervorgerufen werden können, weisen darauf hin, daß diese Veränderungen, aber auch die Vergrößerung der Nebenschilddrüsen durch die gesteigerte Funktion des HVL bedingt werden.

7. Die gesteigerte Funktion des HVL ist, unserer Ansicht nach, nicht auf die spezifische Wirkung der verwendeten Verbindungen zurückzuführen, sondern vielmehr auf die von ihnen ausgelöste, periodische acidotische Einwirkung von mäßigem Grade.

8. Unsere Untersuchungen bieten einen neuen Beweis dafür, daß die Funktion des innersekretorischen Drüsensystems durch entsprechende Verabreichung einfacher, acidotisch wirkender Verbindungen umgestellt bzw. gesteigert werden kann. Auf Grund unserer Ergebnisse steht es zu hoffen, daß gegebenenfalls die Funktion der vermindert

funktionierenden Nebenschilddrüsen (und andrer innersekretorischen Drüsen) wiederhergestellt bzw. normal gemacht und zu gleicher Zeit die durch diese unzulängliche Funktion bedingten pathologischen Zustände behoben werden können.

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Hardy-'29
Bib No. 154

THE RÔLE OF HYDROCHLORIC ACID IN THE CAUSATION OF GASTRIC PAIN.*

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It would seem almost a task of supererogation to revive discussion of an acid factor in the causation of gastric pain, in view of the apparently conclusive observations of Hurst [1] some 20 years ago, observations which have since received confirmation from many sources, clinical and experimental. Nevertheless it cannot be denied that there still exists in the minds of most laymen, and of not a few medical men, the belief that gastric pain must in some way be associated with gastric acidity, a belief which finds its expression in the occasional use of such terms as hyperchlorhydria and acid dyspepsia to denote clinical states illustrating cause and effect, and in the use of sodium bicarbonate or other alkali for their relief. But while the evidence, both clinical and experimental, is definitely opposed to such a view, recent work by Palmer [2] has renewed interest in the subject and certainly demands critical consideration. After confirming Hurst's observations that the mucous membrane of the stomach in healthy individuals was insensitive to concentrations of hydrochloric acid up to 0·5 per cent., he was able to reproduce in ulcer patients under suitable conditions of susceptibility their characteristic pain, not only with 0·5 per cent. hydrochloric acid, but also with concentrations which fell within physiological limits.

The reinjection of the gastric contents obtained at the time of the distress also reproduced it in some instances, and relief was invariably obtained either by emptying the stomach or by the use of alkalis. He found that the pain was produced most readily during the stage of active symptoms, and was usually absent during quiescent periods and after a course of treatment. Further, from a study of 15 cases he found no evidence of a positive response to acid in the absence of an organic lesion of the stomach or duodenum. He finally concluded that hydrochloric acid was not only an adequate stimulus to the pain-producing mechanism of a sensitive peptic ulcer, but was also the stimulus usually in evidence.

It was evident that if these results could be substantiated and the specificity of the test for ulcer established, the clinician would have an additional valuable method of distinguishing gastric pain due to peptic ulcer from that due to distant foci of irritation—for example, in the appendix, the colon, or the gall-bladder. In a later paper Palmer [3] claimed that his methods provided such a test, and the present investigation which follows very much the lines he adopted was undertaken in order to exploit this so-called acid test as an aid in differential diagnosis, and perhaps to throw additional light on the relationship of gastric acidity to gastric pain.

Technique of the Test.

After a night's fast the patient swallows a Ryle's tube and the stomach is emptied of its resting contents. 200 c.cm. of 0·5 per cent. HCl are then introduced into the stomach through the tube. The patient is kept in ignorance of the nature of the injected fluid and is informed that in all probability he will experience no unusual sensation, but that if he does he is to notify the observer. Suitable literature is provided to divert the mind, and at the end of half an hour, if no pain has been complained of, the stomach is emptied and a further 200 c.cm. of acid are introduced, and this is repeated a third time if necessary. The method is substantially that of Palmer, with the slight variation that the stomach is emptied at the end of each half hour period in order to avoid the accumulation of large quantities of fluid and to maintain the concentration of acid at a high level. The test is regarded as positive if the patient complains of unequivocal pain which he is able to recognise as identical with his previous symptoms, identical, that is, in kind, but not necessarily in degree. Severe pain can be promptly relieved by emptying the stomach or by the use of an alkaline powder.

Results of Investigation.

The total number of cases investigated, the great majority of whom attended my out-patient clinic in the first instance, is 228, and the number of examinations carried out 257. They are divisible into two groups—one consisting of 155 cases in whom organic disease of the stomach or duodenum was demonstrable, and the remainder, 73 in number, in whom no local lesion could be discovered. Reliance was placed on X ray diagnosis in 176 cases, severe hæmorrhage in 6, and surgical intervention in 46.

A comparison of the results obtained in the two groups makes it possible to assess the value of the acid test in differential diagnosis.

TABLE I.—*Organic Diseases of Stomach and Duodenum.*

Diagnosis.	Total cases.	Total tests.	Results of acid test.	
			Pos.	Neg.
Duodenal ulcer	87	105	41	64
Gastric ulcer	50	59	35	24
Gastro-jejunal ulcer	4	4	0	4
Gastric carcinoma	3	3	2	1
Hæmatemesis and melæna ..	6	6	1	5
Gastritis..	7	7	3	4
	157	184	82	102

Table I. shows the results obtained from 184 tests carried out on 157 patients suffering from organic disease of the stomach or duodenum. Eighty-two gave positive tests and 102 negative. Peptic ulcer accounts for the great majority of cases, and it will be noted that the proportion of positive results is considerably higher in ulcers of the lesser curvature than of the duodenum. In three cases of carcinoma two were positive, in one of which the pain was agonising, as was also the case in one of the three positive results

* An abstract of this paper was contributed to the discussion on various aspects of gastric chemistry at the Section of Medicine, Royal Society of Medicine, reported on p. 718.

in the cases of gastritis. It is interesting to note that the localisation of the pain, though usually referred somewhat vaguely to the epigastrium, may, when at all severe, be exceedingly accurate and even felt in the back. In one case, that of a duodenal ulcer, pain was, curiously enough, felt only in the left axilla; this localisation was reproduced with great accuracy as a result of a positive acid test.

TABLE II.—*Analysis of 103 Cases of Peptic Ulcer.*

Type.	Cases.	Tests.	Acid +.	Symptoms.		Acid −.	Symptoms.	
				Active.	Quiescent.		Active.	Quiescent.
Duodenal	70	88	37	36	1	51	12	39
Gastric	33	41	24	24	0	17	5	12
	103	129*	61	60	1	68	17	51

Table II. shows the relationship of the results obtained to the period of the disease in which the test was carried out—whether one of active symptoms or of quiescence. Symptoms were considered to be active when spontaneous pain had been present within the preceding 24 hours. Of the 103 cases that are analysed it will be noted that all the positive results, with one exception, were obtained during a period of active symptoms, but more noteworthy is the occurrence of 17 negative tests during the active period. In one of these, a case of lesser curve ulcer, the symptoms were of recent duration, the pain of unusual intensity, and associated with slight rigidity and a localised area of superficial soreness, so that leakage was suspected.

TABLE III.—*Functional Dyspepsia and Extragastric Lesions.*

Diagnosis.	Total cases.	Total tests.	Results of acid test.	
			Pos.	Neg.
Functional dyspepsia	44	45	4	41
Constipation	12	12	2	10
Chronic appendicitis	7	8	4	4
Chronic cholecystitis	5	5	4	1
Cirrhosis of liver	1	1	—	1
Pancreatitis	1	1	—	1
Adhesions	1	1	—	1
	71	73	14	59

Table III. shows the results obtained in 71 cases where a lesion of the gastric mucosa could be excluded. Forty-four were cases of simple functional disturbance with negative X ray picture, and the remainder examples of gastralgia produced by irritative foci acting at a distance. The 12 cases of appendicitis and cholecystitis were confirmed by operation and the stomach and duodenum found normal in each instance. Nevertheless, four out of seven cases of appendicitis, and four out of five of cholecystitis gave positive acid tests, the pain in the latter being severe and in one case of appendicitis exceptionally so.

Thirty-four cases of ulcer were submitted to operation, and I have to thank my surgical colleague, Mr. H. H. Sampson, under whom the patients were admitted, for permission to make use of this material from which it is possible to obtain a clearer conception of the state of the ulcer in relation to the acid response. The acid test was found to be negative in 20 cases in whom little or no evidence of healing was observed at the operation.

As regards the results of treatment, 24 cases of ulcer with positive results were examined at the end of a course of medical treatment. In all but one the test was negative, and that was a case of a large active ulcer associated with an hour-glass constriction of the stomach. How soon the test becomes negative I am not prepared to say, but I have found it so a week from the commencement of treatment. One test was carried out a week after gastro-jejunostomy and was found to be negative, and a negative result was present a week after removal of the appendix; in both cases the test had previously been positive.

Discussion.

As a result of this investigation I have attempted to supply answers in part or in whole to two questions: (1) Is there an acid response sufficiently specific for ulcer cases to constitute a test of diagnostic value? (2) What part does hydrochloric acid play in the production of gastric pain?

The answer to the first question must clearly be in the negative, for pain typical in every way of ulcer, both as regards severity and localisation, was forthcoming, though not so commonly, in cases where a lesion of the gastric mucosa could undoubtedly be excluded (Table II.). General considerations would suggest that this must be so for the pains of ulcer can be simulated with absolute fidelity by a number of extra-gastric lesions, and must therefore almost certainly share with them a common mechanism. The discrepancy between the results described in this paper and those of Palmer can only be explained on the assumption that his cases were either examined during a period of quiescence or, more probably, were too few to bring out this point, for his series contains only one example each of chronic cholecystitis and subacute appendicitis, the lesions most likely to simulate ulcer in their symptomatology.

As regards the second question—the rôle of hydrochloric acid in the production of gastric pain—the matter is more difficult, and I am not prepared with a satisfactory answer. There seem to be three possible ways in which a chemical irritant might act: (1) by irritating the exposed nerve-endings in an area of ulceration; (2) by causing local spasm at the ulcer site and thus interfering with normal peristalsis; or (3) by causing a primary increase of tonus, as recently suggested by Poulton.[1]

The first two possibilities may be largely discounted, for in addition to being generally discredited on experimental grounds they fail to account for pain in the absence of structural defect. That there may be a local factor in certain instances is, however, suggested by some work of Ivy privately communicated to Alvarez,[5] which suggests that though not normally sensitive to painful stimuli, a mucous membrane, the seat of œdema or congestion, may become so.

The production of an increase in gastric tonus, and so pain, by hydrochloric acid lacks proof. All that can be said at the moment is that gastric pain typical in every way of ulcer has been produced by a number of experimenters, notably Poulton[4] and Hardt,[6] without calling in an acid factor; Hardt indeed in one case of gastric ulcer actually brought about relief by the introduction of 0·3 per cent. hydrochloric acid.

Palmer regarded hydrochloric acid as the adequate stimulus for ulcer pain normally present in the gastric juice; that it may be adequate in supernormal concentrations and at times in physiological ones he has abundantly proved, and his results have been confirmed in this paper, but when in his final summary[7] he states that acid is the normal stimulus for the pain mechanism I find it impossible to follow him, for my investigations so far as they go are definitely opposed to such a view. In the first place, the pain is rarely severe, and the patient, though able to identify it, will often volunteer the information that it is not nearly so severe as his typical distress, and this, be it noted, in the presence of acid values far in excess of those ever present in health or disease. The pain rarely shows itself when the acid concentration is at its maximum, that is, immediately after its introduction. Frequent analyses show that the acidity has usually undergone some reduction, at times considerable, before complaint is made. It is possible to explain this latent period on the grounds of a diminished threshold, but this is by no means always borne out by the patient's symptoms, and I have recounted one

case of gastric ulcer of quite exceptional severity with a negative acid test.

With the weaker solutions, 0·25 per cent., I have frequently failed to get a satisfactory response when it was readily provoked by the stronger, and in the following experiment the reintroduction of gastric contents failed to reproduce pains of anything like the severity with which they were previously associated, and a subsequent injection of 0·5 per cent. HCl was practically without effect.

CASE R. B.—Big lesser curve ulcer; gastro-jejunostomy previously.

	Fasting juice, 15 c.cm.	Free HCl. 14 c.cm.*
9.45 :	200 c.cm. tap water.	
10.15 :	10 c.cm. only left	5 "
	200 c.cm. 0·5 per cent. HCl.	142 "
10.20 :	Pain severe.	
10.30 :	Stomach emptied 250 c.cm.	64 "
	Pain went at once.	
10.50 :	Contents 250 c.cm. reintroduced.	
11.10 :	Pain very slight indeed.	
11.20 :	Pain gone.	
11.50 :	Stomach emptied 35 c.cm.	−12 "
11.50 :	100 c.cm. 0·5 per cent. HCl.	142 "
12.5 :	Pain slight.	
12.15 :	Stomach emptied 105 c.cm.	65 "
12.20 :	Pain much easier.	

* 1/10 N. soda per cent.

It might have been expected if hydrochloric acid were the normal stimulus for pain production that some relationship would be found between the type of pain the patient complained of, whether early or delayed after meals, and the latent period following the acid test, but no such relationship could be established, the proportion of positive results occurring during the first and second periods being roughly the same in the 47 cases in which this point was specifically noted. (Table IV.)

TABLE IV.

Type of pain.	No. of positives.	Period of test.		
		First.	Second.	Third.
Early ..	20	12	7	1
Delayed ..	27	17	9	1
	47	29	16	2

The use of histamine naturally suggests itself as a means of adding confirmation to the acid theory, but I have so far failed to produce any sensation whatsoever in ten ulcer patients, previously acid positive and still in an active phase, by the injection of one milligramme of histamine acid phosphate, though the subsequent acid values were well above normal; and in a recent experiment a failure with histamine was immediately followed by a positive result with 0·25 per cent. of HCl, though the acid values were at a considerably lower level.

CASE W. B.—Duodenal ulcer.

	Fasting juice 25 c.cm.	Free HCl. 10 c.cm.*
Raw :	1 mg. histamine acid phosphate	
10 mins. :	Stomach emptied 26 c.cm.	32 "
17 "	" " 35 "	70 "
24 "	" " 62 "	95 "
44 "	" " 21 "	89 "
60 "	" " 15 "	83 "
	200 c.cm. 0·25 per cent. HCl.	
60 mins. :	Pain began.	
65 "	Pain fairly severe. Specimen	74 "
74 "	Pain easier.	46 "
77 "	Pain almost gone.	
90 "	" " "	
92 "	Stomach emptied 100 c.cm.	37 "
98 "	Pain still just appreciable.	
	200 c.cm. 0·5 per cent. HCl.	
	Pain at once.	
100 mins. :	Pain worse	137 "
120 "	Pain still severe. Specimen	51 "
	One drachm magnesium phosphate.	
115 mins. :	Pain easier	32 "
117 "	Pain gone	67 "

* 1/10 N. soda per cent.

It may be that the volume of the injected fluid plays a part, but I have no evidence to show that this is so, and I recall two cases in which the pain increased in severity after the stomach had been emptied.

Clinical observation from many aspects tends to support these findings and to suggest that the pains of peptic ulcer are independent of acid concentrations, for it is well recognised that severe and typical distress may be associated, though not commonly, with achlorhydria. The rapid relief of pain which follows the institution of an ulcer régime, whether associated with intensive alkalinisation as in Sippy's methods or following those of Lenhartz and Smithies in which alkalis play no essential part, is rarely accompanied by any lowering of acid values, and the present author[8] concluded that clinical improvement was independent of chemical change in a considerable number of cases, while in a few it was actually associated with a rise in acidity. Crohn and Reiss,[9] as a result of an intensive study of 34 ulcer cases treated by Sippy's methods, found a rough parallelism only between clinical and chemical improvement.

The view that alkalis relieve gastric pain by a process of neutralisation must also be subject to criticism, for it is noteworthy that such a result may be equally prompt in cases where the acid values are low, and indeed in some cases of carcinoma where free acidity is absent. Fractional studies, too, show that such neutralisation is of very brief duration, and is usually followed by a rise in acidity in excess of that previously recorded; yet the clinical relief outlasts the period of neutralisation and is unaffected by the subsequent hyperacidity. The explanation of the beneficial action of alkalis is probably that advanced by Spencer, Meyer, Rehfuss, and Hawke,[10] who found that these substances brought about a more rapid emptying of the stomach.

Such evidence, then, as I have been able to bring forward in this paper is opposed to any view which assigns to hydrochloric acid anything but a very minor rôle in the mechanism of gastric pain. The vagaries of gastric pain are well known. It comes and goes in a manner which suggests little dependence on chemical factors. The internal influences, infection, fatigue, tobacco, and the external ones, worry, rest, change, speak for a nervous rather than an acid control, and while it cannot be denied that the gastric acidity may occasionally be effective it must be emphasised that no theory of gastric pain can be regarded as satisfactory unless it will explain all the facts.

Conclusions.

1. Hydrochloric acid in concentrations of 0·5 per cent. will reproduce typical gastric pain in certain susceptible individuals suffering from painful disturbances of gastric function.

2. The pain occurs independently of any local disease in the stomach or duodenum.

3. The rôle of hydrochloric acid is discussed and the view put forward that it plays no essential part in ordinary pain production.

I gratefully acknowledge the valuable help I have received from Miss Fisher and Miss Graham, nursing sisters, in carrying out the tests, and from Mr. Garfield Thomas, biochemist to the hospital, who undertook numerous chemical analyses.

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Hermann-'38
Bib No. 164

EFFECTS AND FATE OF ACIDS IN THE ORGANISM. I. REPORT: DETERMINATION OF HYDROGEN ION CONCENTRATION IN THE BLOOD STREAM OF RABBITS AFTER INTRAVENOUS INJECTION OF FREE ACIDS (Saurewirkungen und Saureschicksal im Organismus. I. Mitteilung: Bestimmung der Wasserstoffionenkonzentration im stromenden Kaninchenblut nach intravenoser Injektion freier Sauren)

By

Siegwart Hermann, Richard Neiger and

Margot Zentner

from

Naunyn-Schumiedeberg's Archiv. Experimentelle Pathologie und Pharmakologie 188: 52b-532, 1938.

Translation from German

EFFECTS AND FATE OF ACIDS IN THE ORGANISM.

I. REPORT: DETERMINATION OF HYDROGEN ION CONCENTRATION IN THE BLOOD STREAM OF RABBITS AFTER INTRAVENOUS INJECTION OF FREE ACIDS

By

Siegwart Hermann, Richard Neiger and Margot Zentner

In earlier tests we have already found that the hydrogen ion concentration increases after intravenous feeding of gluconic acid. From various effects after peroral introduction of gluconic acid, we were also able to conclude that an increase occurs in the hydrogen ion concentration in the organism. Thus, symptoms of sclerosis occurring from Vitasterin could be prevented and partly even eliminated by the use of Kombuchal which contains 30% free gluconic acid. Also the change of the calcium quotient, i.e. the increase of dissociated calcium in the serum can be traced back to the hydrogen ion concentration. Gluconic acid belongs to the poorly combustible acids, and it is therefore readily understandable that it can shift the pH in the organism to the acid side. However, there are also acids which behave in the opposite way, e.g. acetic acid which for various reasons is considered an easily "combustible" acid. The results of some of our tests could not be always reconciled with prior conceptions. We therefore decided to systematically study a larger number of acids.

This publication deals exclusively with the changes in the hydrogen ion concentration in the blood after introduction of free acids (not salts). After considering various methods for measuring the hydrogen ion concentration, we decided for the intravenous von Brehmer method since it

appeared to us from the publication of Bucheler, Zucker and Capaldi, Wunsche, as well as from our own comparative tests, that this method yields very suitable results for comparable determinations.

METHODOLOGY

Rabbits of an average individual weight of 2000 g were put on an oats-water diet prior to the start of testing. For the execution of the test it is important that the animals be prevented from coming in contact with metallic objects. If necessary, clamping devices have to be wrapped with insulating tape. The rabbit is strapped to a wooden board and its right front paw stuck through an opening in the board and immobilized. After removal of hair and grease from the paw, the latter is dipped into a vessel containing a saturated potassium chloride solution. The vessel in turn is conductively connected via a tube filled with potassium chloride agar to the measuring vessel of an ionometer (Lautenschlager). The antimony-hemovein needle is introduced into the peripheral ear vein of the rabbit, thereby closing the circuit. The useability of the hemovein needle is tested by means of solutions of known pH and is also already indicated by the testing of the rabbit's normal pH values. Occasionally, a sudden drop in pH occurs. If this is merely due to the formation of a thrombus, correct values will again be obtained after removal of the thrombus. Otherwise the hemovein needle has to be replaced by another one. The rectal temperature of the rabbit was taken into consideration in correcting the pH values. Those pH values that remained constant 1/2 hour before the injection were considered normal. The acid was injected into the vena jugularis dextra through a hollow needle with a

shut-off cock. In the course of 3 minutes, 10 cm^3 of the respective acids were injected, followed by 10 minutes of measuring the pH of the flowing blood, then another injection etc. Below we describe by means of graphs the results of our measurements after injection of various acids. The observation time was about 6-8 hours. Although 4-6 animals were used for each acid, we always show, for the sake of brevity, only one of the curves obtained, provided it was found to be essentially very similar to the remaining curves.

RESULTS OF THE pH MEASUREMENTS AFTER INTRAVENOUS INTRODUCTION OF FREE ACID INTO FLOWING BLOOD

1. *Phosphoric acid*

n/2 phosphoric acid with a pH of 1.45 was injected. It is lipide-soluble. The lethal dose is 62.3 cm^3 of n/2 acid or 1 g H_3PO_4 per kg rabbit. The pH drop amounts on an average to 0.58. Death occurs suddenly, accompanied by dyspnea and spasms in the tensor muscles. Attempts to save the animals immediately after occurrence of the first symptoms of intoxication by intravenous injection of sodium bicarbonate resulted in failure. The effect of the acid on the flowing blood in the individual phases can be seen from curve 1 in Fig. 1.

2. *Gluconic acid*

The effect of n/2 gluconic acid is to lower the pH by an average of 0.46. The pH of the n/2 acid is 2.05. Gluconic acid is insoluble in lipides. Substantial amounts of gluconic acid are tolerated both orally and parenterally. In our tests the lethal dose was never reached. The animals received intravenously 6 g or 61 cm^3 of n/2 gluconic acid per kg.

With these quantities, dyspnea occurred, but was alleviated by injection two to three times of 10 cm^3 of n sodium bicarbonate solution. The blood pH also returned to normal. After removal of the hollow pin, the animals still lived for several days. (Fig. 1, curve 2).

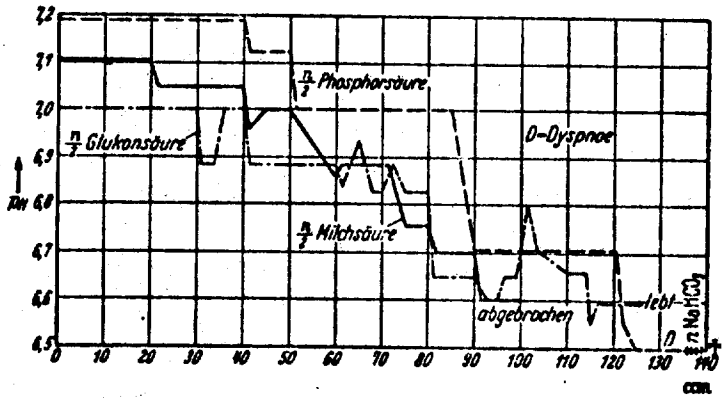

Abb. 1.

Fig. 1.

3. Lactic acid

Lactic acid n/2 with a pH of 1.90 distinctly acidifies the blood. It is tolerated by the rabbit in relatively large quantities. It is lipide-soluble. The toxic dose is 1.76 kg per kg animal, the lethal dose 2.01 g or 80 cm^3 of n/2 acid per kg. (Fig. 1, curve 3).

4. Tartaric acid

n/2 acid of pH 1.8 was injected. The average drop in pH after an intravenous injection amounts to 0.4. Tartaric acid is lipide-soluble. It is said to be incombustible. The lethal dose was 1.1 g or 30 cm^3 n/2 tartaric acid per kg rabbit. Tensor cramps did not occur until just before death. (Fig. 2, curve 1).

5. Hydrochloric acid

n/4 hydrochloric acid was injected. Its pH is less than 1.0. It is lipide-soluble and non-combustible. The largest amount that can be injected per kg rabbit until death occurs is 0.6 g or 54.2 cm^3 of n/4 hydrochloric acid. The pH is lowered by an average of 0.35, and the hydrogen ion concentration is raised accordingly. Death occurs with the same symptoms as in the case of phosphoric acid. Injections of sodium bicarbonate as an antitoxin were unsuccessful. (Fig. 2, curve 2).

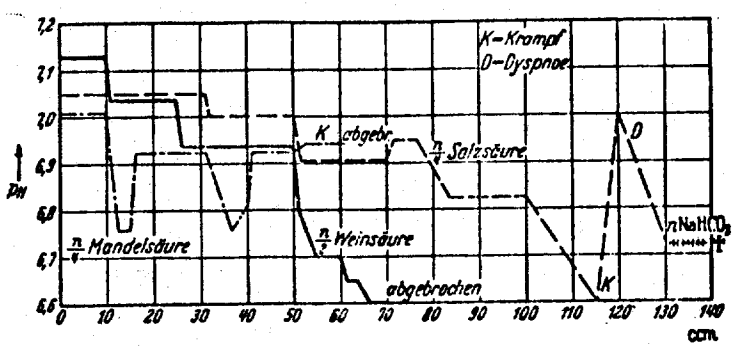

Abb. 2.

Fig. 2.

6. Mandelic acid

n/4 solutions with a pH of 2.05 were injected. The average pH drop amounted to 0.25. Mandelic acid is lipide-soluble and incombustible in the organism. Its strong toxicity is remarkable. The lethal dose is 1.65 g or 15.5 cm^3 of n/4 solution per kg rabbit. n/2 solutions could not be injected at all in view of their great toxicity. The effect on the pH can be seen in Fig. 2, curve 3.

7. Acetic acid

After intravenous injection of n/4 acetic acid, a distinct alkalization of the blood can be observed. The blood pH is raised by an average of 0.5. This increase is due to the formation of sodium acetate. The pH of n/2 sodium acetate solution is 7.3. After injection of 50 cm^3 of n/4 acetic acid, a steep drop in the blood pH occurs. Soon after that, the animals died, with dyspnea and spasms. By this time the acetic acid had bound the available alkali reserve, so that a surplus of free acetic acid soon leads to death. The spontaneously discharged urine is bloody. The measurement of the urine-pH during the experiment showed a slight rise from 5.3 to 5.45. The acetic acid is lipide-soluble, the pH of the n/4 acid amounts to 2.70. The lethal dose is 0.5 g CH_3COOH or 33.5 cm^3 of n/4 solution per kg animal. (Fig. 3, curve 1).

8. Maleic acid

n/2 solutions with a pH of 1.50 n/4 acid with a pH of 1.60 and n/8 acid with a pH of 1.70 were injected. The n/2 acid is extremely toxic. After injection of the n/2 acid, the pH dropped rapidly, but then soon rose somewhat. At this moment, death occurred. After injection of the n/4 acid, the pH rises appreciably, then soon returns to its original value and after that goes up again strongly. Only shortly before death, accompanied by dyspnea and spasms, the blood-pH drops to almost normal. Alkalization becomes very obvious after injection of the n/8 acid. Maleic acid is lipide-soluble. The lethal dose for the n/8 solution is 0.19 g maleic acid or 26 cm^3 per kg animal. (Fig. 3, curve 2).

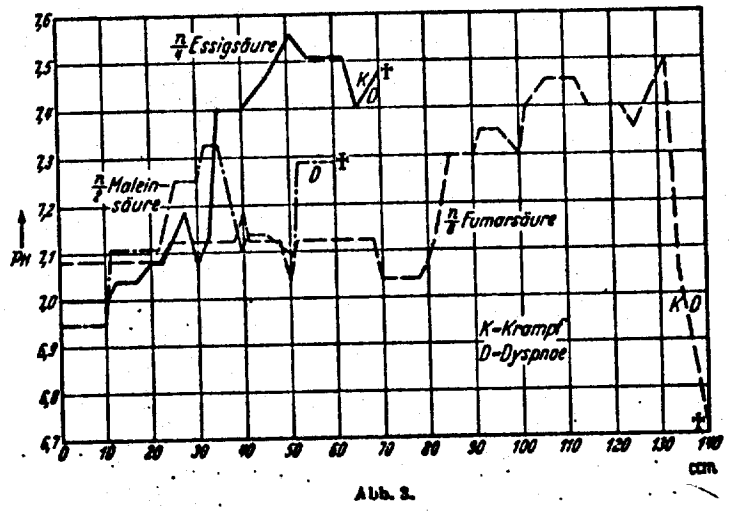

Fig. 3.

9. Fumaric acid

Fumaric acid is a structural isomer of maleic acid. Curve 3 in Fig. 3, which represents the course of injections with the n/8 acid, shows a striking difference from the curve of maleic acid. One can inject 70 cm^3 fumaric acid without causing any change in the pH. Only after that does the pH rise fairly steeply until, before death, it drops rapidly below normal. n/8 fumaric acid has a pH of 2.25. It is lipide-soluble. The lethal dose of n/8 fumaric acid is 0.47 g per kg animal, which is more than twice the figure for maleic acid.

10. Malic acid

n/4 malic acid, which has a pH of 2.20 in four different injection tests, displayed a varying curve shape. Two tests showed an increase, two a decrease in the pH. This may possibly be explained by the transformability of this acid in the organism. It is also possible that the malic acid, being a dibasic acid, forms both acid and neutral salts in the

blood, so that the reaction is determined by the one or the others. The solubility of malic acid in lipides is poor. The lethal dose of the n/4 acid is 2.40 g or 106 cm^3 per kg animal. (Fig. 4).

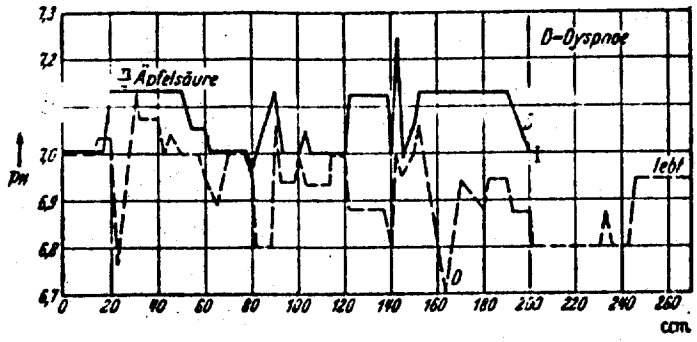

Abb. 4.

Fig. 4.

11. Succinic acid

There is a pronounced difference in tolerance depending on whether n/4 or n/2 acid has been injected. Over 200 ccm of n/4 were tolerated, whereas a maximum of 50 cm^3 of the n/2 acid could be injected. The shape of the curves has something in common with that of malic acid (the two are chemically closely related), namely the zig-zag pattern. The pH values fluctuate around the initial value. As in the case of malic acid, it is possible that a partly acid salt and a partly neutral salt are formed, so that no uniform direction in the pH change can occur. Succinic acid is insoluble in lipides. Of the n/2 acid, 0.54 g or 18.1 cm^3 were tolerated per kg animal, of the n/4 acid, 1.63 g or 88.0 cm^3. (Fig. 5, curve 1).

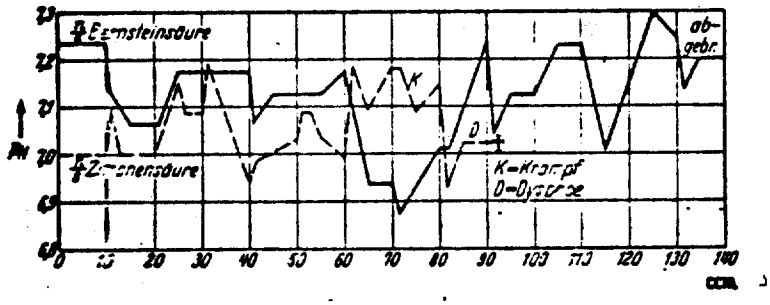

Fig. 5.

12. Citric acid

n/4 and n/8 solutions were injected. The pH of the n/4 acid is 2.15, that of the n/8 acid 2.3. The solubility in lipides is poor. In case of oral introduction the acid, according to Langecker, is combustible. The lethal dose for the n/4 acid is 0.28 g or 17.10 cm^3, for the n/8 acid 0.22 g or 32.90 cm^3 per kg animal. Fig. 5, curve 2 shows the tendency of temporary alkalization. The pH temporarily rises, and the hydrogen ion concentration drops correspondingly.

ABSTRACT

1. The change in the hydrogen ion concentration in flowing venous blood was determined after intravenous injection of various acids.

2. Three basic groups of acids can be distinguished: Those which acidify the blood, those which alkalize it, and finally those which manifest effects in both the acid and the alkaline directions.

3. In the order of blood acidification capacity, the sequence is as follows: Phosphoric acid > gluconic acid > lactic acid > tartaric acid > hydrochloric acid > mandelic acid.

4. In the order of blood alkalization capacity, the sequence is as follows: Acetic acid > maleic acid > fumaric acid.

5. Both acidification and alkalization occur with malic acid; succinic acid shows temporary effects toward the acid side, citric acid toward the alkaline side.

Hermann-'38
Bib No. 164

Arch. Exptl. Path. Pharmakol. 188:526-532. 1938.

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Säurewirkungen und Säureschicksal im Organismus.

I. Mitteilung: Bestimmung der Wasserstoffionenkonzentration im strömenden Kaninchenblut nach intravenöser Injektion freier Säuren.

Von

Siegwart Hermann, Richard Neiger und **Margot Zentner.**

Mit 5 Textabbildungen.

(Eingegangen am 15. Dezember 1937.)

In früheren Untersuchungen[1] haben wir bereits festgestellt, daß sich die Wasserstoffionenkonzentration nach intravenöser Zufuhr von Gluconsäure erhöht. Aus verschiedenen Wirkungen nach peroraler Zufuhr von Gluconsäure konnten wir auch den Schluß ziehen, daß eine Zunahme der Wasserstoffionenkonzentration im Organismus stattfindet. So konnten mit Kombuchal, welches 30% freie Gluconsäure enthält, Verkalkungserscheinungen nach Vitasterin verhindert, ja zum Teil sogar rückgängig gemacht werden[2]. Auch die Veränderung des Calciumquotienten[3], d. h. die Zunahme von dissoziiertem Calcium im Serum kann auf Änderung der Wasserstoffionenkonzentration zurückgeführt werden. Die Gluconsäure gehört zu den schwer verbrennbaren Säuren und es ist infolgedessen leicht einzusehen, daß sie im Organismus das p_H nach der sauren Seite verschieben kann. Es gibt aber auch Säuren, die ein entgegengesetztes Verhalten zeigen, z. B. die Essigsäure, die man aus verschiedenen Gründen zu den leichter „verbrennbaren" Säuren rechnet. Bei unseren Untersuchungen ergaben sich Befunde, die mit den bisherigen Vorstellungen nicht immer in Einklang zu bringen waren. Aus diesem Grunde haben wir uns entschlossen eine größere Zahl von Säuren systematisch zu untersuchen. Die vorliegende Arbeit soll sich ausschließlich mit den Änderungen der Wasserstoffionenkonzentration im Blute nach Zufuhr von freien Säuren (nicht Salzen) befassen. Bei der Auswahl der Methode zur Messung der Wasserstoffionenkonzentration haben wir uns für die intravitale Methode von v. Brehmer[4]

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[1] Hermann, Siegwart: Naunyn-Schmiedebergs Arch. **154**, 144—192 (1930). — [2] Hermann, Siegwart: Klin. Wschr. **1929**, Nr. 38, S. 1752—57. — [3] Hermann, Siegwart u. Margot Zentner: Naunyn-Schmiedebergs Arch. **175**, 500 (1934). — [4] v. Brehmer: Sonderdruck aus dem Paracelsus Inst. Schriftenreihe des Vereins Deutsche Volksheilkunde, 2.

entschieden, da wir sowohl aus den Arbeiten von Bücheler, Zucker und Capaldi[5], Wünsche[6] als auch aus eigenen Vergleichsmessungen den Eindruck gewonnen haben, daß diese Methode für vergleichende Bestimmungen sehr brauchbare Resultate liefert.

Methodik.

Kaninchen im Gewichte von durchschnittlich 2000 g wurden vor Versuchsbeginn auf Hafer-Wasserkost gesetzt. Für die Durchführung des Versuchs ist es wichtig, daß die Tiere nicht mit Metallteilen in Berührung kommen. Erforderlichenfalls sind Klemmvorrichtungen mit Isolierband zu umwickeln. Das Kaninchen wird auf einem Holzbrett aufgespannt, die rechte Vorderpfote durch eine Öffnung im Brett durchgesteckt und fixiert. Die von Haaren und Fett befreite Pfote wird in ein Gefäß mit gesättigter Kaliumchloridlösung getaucht. Dieses Gefäß wird durch eine mit Kaliumchloridagar gefüllte Röhre mit dem Meßgefäß des Ionometers (Lautenschläger) leitend in Verbindung gebracht. Die Antimon-Hämovennadel wird in die Ohrrandvene des Kaninchens eingeführt und dadurch der Stromkreis geschlossen. Die Brauchbarkeit der jeweils verwendeten Hämovennadel wird an Lösungen mit bekanntem p_H geprüft und ergibt sich auch schon aus der Prüfung der normalen p_H-Werte des Kaninchens. Bisweilen kommt es zu einem plötzlichen Abfall des p_H. Ist nur eine Thrombenbildung daran schuld, so erhält man nach Entfernung des Thrombus wieder einwandfreie Werte. Anderenfalls ist die Hämovennadel durch eine andere zu ersetzen. Die rektale Temperatur des Kaninchens wurde bei der Korrektur der p_H-Werte berücksichtigt. Als normale p_H-Werte wurden diejenigen angesehen, die vor der Injektion $^1/_2$ Stunde konstant blieben. Die Injektion der Säure erfolgte durch eine Hahnkanüle in die Vena jugularis dextra. Im Verlauf von 3 Minuten wurden 10 ccm der betreffenden Säure injiziert, hierauf durch 10 Minuten das p_H des strömenden Blutes gemessen, wieder injiziert usw. Im nachstehenden bringen wir die Ergebnisse unserer Messungen nach Injektion verschiedener Säuren an Hand von Kurven. Die Beobachtungsdauer betrug ungefähr 6—8 Stunden. Obwohl für jede Säure 4—6 Tiere verwendet wurden, veröffentlichen wir der Kürze halber nur je eine der erhaltenen Kurven, für den Fall, daß sie im wesentlichen übereinstimmen.

Ergebnisse der p_H-Messungen nach intravenöser Zufuhr freier Säuren im strömenden Blute.

1. Phosphorsäure.

Injiziert wurde n/2 Phosphorsäure von einem p_H 1,45. Sie ist lipoidlöslich. Die tödliche Dosis beträgt 62,30 ccm n 2 Säure bzw. 1 g H_3PO_4 pro kg Kaninchen. Die p_H-Erniedrigung beträgt im Mittel 0,58. Der Tod erfolgt plötzlich unter Auftreten von Dyspnoe und Krämpfen der Streckmuskulatur. Versuche, sofort nach Eintreten der ersten Vergiftungssymptome Tiere durch intravenöse Injektion von Natriumbicarbonat zu retten, schlugen fehl. Der Einfluß der Säure auf das strömende Blut ist in den einzelnen Phasen aus der Kurve 1, Abb. 1 zu ersehen.

[5] Zucker u. Capaldi: Radiologische Rundschau VI. 1937. — [6] Wünsche, Otto: Klin. Wschr. 1936. Nr. 31. S. 1102.

2. Gluconsäure.

Durch n/2 Gluconsäure wird das p_H im Durchschnitt um 0,46 erniedrigt. Das p_H der n/2 Säure beträgt 2,05. Gluconsäure ist lipoidunlöslich. Von Gluconsäure werden sowohl oral als auch parenteral sehr

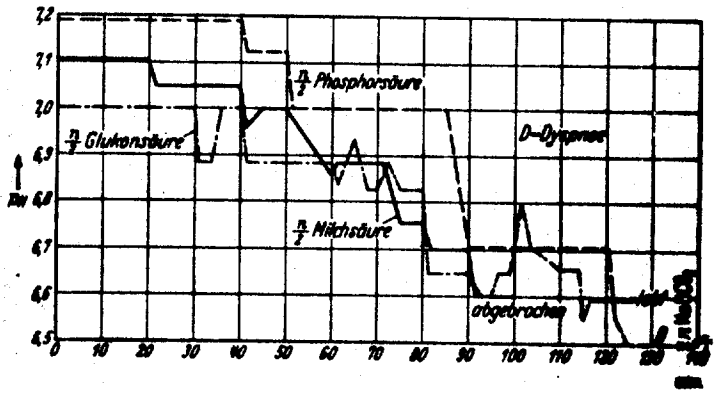

Abb. 1.

große Mengen vertragen. In unseren Versuchen wurde die letale Dosis überhaupt nicht erreicht. Die Tiere erhielten 6 g bzw. 61 ccm n/2 Gluconsäure pro kg intravenös. Bei diesen Mengen trat allerdings bereits Dyspnoe auf, die aber durch Injektion von zwei- bis dreimal 10 ccm einer n Natriumbicarbonatlösung aufgehoben wurde. Auch das Blut-p_H kehrte wieder zur Norm zurück. Die Tiere lebten nach Entfernung der Kanüle noch mehrere Tage (Abb. 1, Kurve 2).

3. Milchsäure.

n/2 Milchsäure, die ein p_H von 1,90 hat, säuert deutlich das Blut. Sie wird in verhältnismäßig großer Menge vom Kaninchen vertragen. Sie ist lipoidlöslich. Die toxische Dosis beträgt 1,76 g pro kg Tier, die letale Dosis 2,01 g bzw. 80 ccm n/2 Säure pro kg (Abb. 1, Kurve 3).

4. Weinsäure.

Injiziert wurde n/2 Säure von p_H 1,8. Die durchschnittliche Erniedrigung des p_H beträgt nach der intravenösen Injektion 0,4. Weinsäure ist lipoidlöslich. Nach oraler Aufnahme soll sie unverbrennbar sein[7]. Die tödliche Dosis betrug 1,1 g bzw. 30 ccm n/2 Weinsäure pro kg Kaninchen. Erst vor dem Tode kommt es zu Streckkrämpfen (Abb. 2, Kurve 1).

5. Salzsäure.

Injiziert wurde n/4 Salzsäure. Ihr p_H ist kleiner als 1,0. Sie ist lipoidlöslich und unverbrennbar. Die größte Menge, die pro kg Kaninchen bis

[7] Underhill, Gross u. Jalesky: J. of Pharm. 43, 359 (1931).

zum Eintritt des Todes injiziert werden kann, beträgt 0,6 g bzw. 54,2 ccm n/4 Salzsäure. Das p_H wird im Durchschnitt um 0,35 gesenkt, die Wasserstoffionenkonzentration dementsprechend erhöht. Der Tod erfolgt unter den gleichen Symptomen wie bei der Phosphorsäure. Injektionen von Natriumbicarbonat als Gegengift sind ohne Erfolg (Abb. 2, Kurve 2).

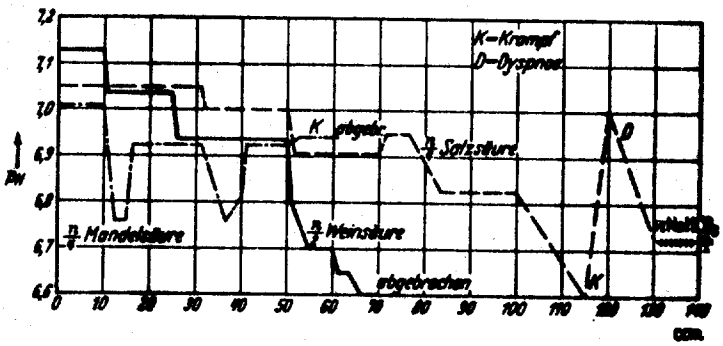

Abb. 2.

6. Mandelsäure.

Injiziert wurden n/4 Lösungen von einem p_H 2,05. Die Durchschnittserniedrigung des p_H betrug 0,25. Mandelsäure ist lipoidlöslich und im Organismus unverbrennbar. Hervorzuheben ist die starke Toxizität. Die tödliche Dosis beträgt 1,65 g bzw. 15,5 ccm einer n/4 Lösung pro kg Kaninchen. n/2 Lösungen konnten wegen der großen Giftigkeit überhaupt nicht mehr injiziert werden. Der Verlauf der p_H-Beeinflussung ist aus der Abb. 2, Kurve 3 zu ersehen.

7. Essigsäure.

Nach intravenöser Injektion von n/4 Essigsäure sieht man eine deutliche Alkalisierung des Blutes. Das Blut-p_H wird durchschnittlich um 0,5 erhöht. Diese Erhöhung ist auf Natriumacetatbildung zurückzuführen. Das p_H einer n/2 Natriumacetatlösung beträgt 7,9. Nach Injektion des 50. ccm von n/4 Essigsäure erfolgte ein steiler Abfall des Blut-p_H. Die Tiere gingen dann unter Auftreten von Dyspnoe und Krämpfen rasch zu Grunde. Die Essigsäure hat in diesem Zeitpunkt die verfügbare Alkalireserve abgebunden, so daß ein Überschuß der freien Essigsäure sehr rasch zum Tode führt. Der spontan entleerte Harn ist blutig. Die Messungen des Harn-p_H während des Versuchs ergaben einen leichten Anstieg von 5,3 auf 5,45. Die Essigsäure ist lipoidlöslich, das p_H der n/4 Säure beträgt 2,70. Die tödliche Dosis ist 0,5 g CH_3COOH bzw. 33,5 ccm einer n/4 Lösung pro kg Tier (Abb. 3, Kurve 1).

8. Maleinsäure.

Injiziert wurden n/2 Lösungen mit einem p_H 1,50; n/4 Säure mit einem p_H 1,60 und n/8 Säure mit einem p_H 1,70. Die n/2 Säure ist außer-

ordentlich giftig. Nach der Injektion einer n 2 Säure fällt das p_H rasch ab um alsbald ein wenig wieder anzusteigen. In diesem Zeitpunkt tritt der Tod ein. Nach Injektion der n 4 Säure steigt das p_H ziemlich an, kehrt alsbald zum Ausgangspunkt zurück um wieder stark anzusteigen. Erst kurz vor dem Tode erfolgt unter Auftreten von Dyspnoe und Krämpfen ein Abfall des Blut-p_H bis fast zur Norm. Nach der Injektion der n/8 Säure ist die Alkalisierung ganz deutlich ausgeprägt. Maleinsäure ist lipoidlöslich. Die letale Dosis beträgt für die n 8 Lösung 0,19 g Maleinsäure bzw. 26 ccm pro kg Tier (Abb. 3, Kurve 2).

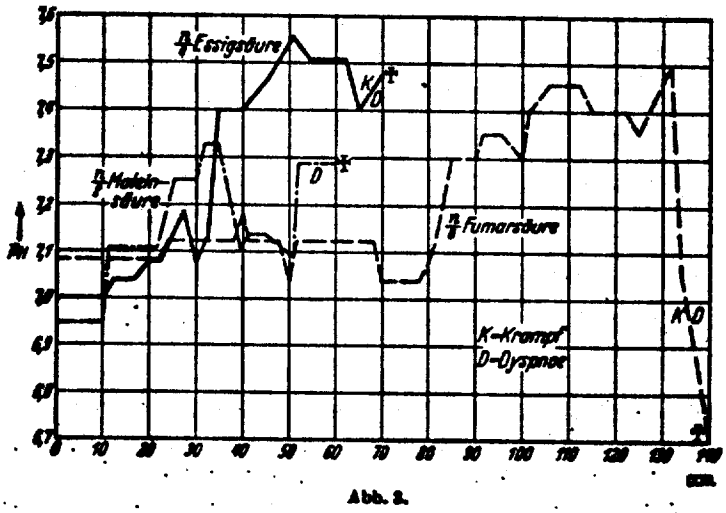

Abb. 3.

9. Fumarsäure.

Die Fumarsäure ist mit der Maleinsäure strukturisomer. Betrachtet man die Kurve 3 der Abb. 3 im Verlauf der Injektionen mit einer n/8 Säure, so fällt der Unterschied gegenüber der Maleinsäurekurve auf. Es können 70 ccm Fumarsäure injiziert werden, ohne daß überhaupt eine p_H-Änderung erfolgt. Erst später steigt das p_H ziemlich steil an, um vor dem Tode sehr rasch unter die Norm abzusinken. n/8 Fumarsäure hat ein p_H von 2,25. Sie ist lipoidlöslich. Die letale Dosis der n/8 Fumarsäure beträgt 0,47 g pro kg Tier, also mehr als das Doppelte der Maleinsäure.

10. Äpfelsäure.

n/4 Äpfelsäure, die ein p_H von 2,20 hat, zeigt in vier verschiedenen Versuchen während der Injektion einen wechselnden Verlauf der Kurven. Zwei Versuche zeigten eine Erhöhung, zwei eine Erniedrigung des p_H. Vielleicht hängt dies mit der Umwandlungsfähigkeit dieser Säure im Organismus zusammen. Es ist auch möglich, daß die Äpfelsäure als zweibasische Säure sowohl saure, als auch neutrale Salze im Blute bildet, so daß

die einen oder die anderen die Reaktion bestimmen. Äpfelsäure ist wenig lipoidlöslich. Die letale Dosis einer n/4 Säure beträgt 2,40 g bzw. 106 ccm pro kg Tier (Abb. 4).

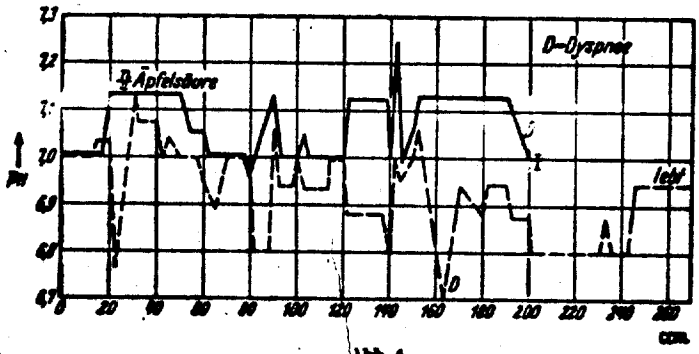

Abb. 4.

11. Bernsteinsäure.

Hervorzuheben ist der große Unterschied in der Verträglichkeit je nachdem ob n/4 oder n/2 Säure injiziert wurde. Von der n/4 Säure wurden über 200 ccm vertragen, während von der n/2 maximal 50 ccm injiziert werden konnten. Der Verlauf der Kurven erinnert insofern an den der Äpfelsäure (die chemische Verwandtschaft ist eine sehr nahe) als auch hier ein Zick-Zack-Verlauf vorliegt. Die p_H-Werte schwanken um den Anfangswert. Bei der Bernsteinsäure dürften sich vielleicht ebenso wie bei der Äpfelsäure teils das saure, teils das neutrale Salz bilden, so daß es zu keiner eindeutigen p_H-Änderung kommen kann. Die Bernsteinsäure ist lipoidunlöslich. Von der n/2 Säure wurden 54 g bzw. 18,1 ccm pro kg, von der n/4 1,63 g bzw. 88,0 ccm pro kg Tier vertragen (Abb. 5, Kurve 1).

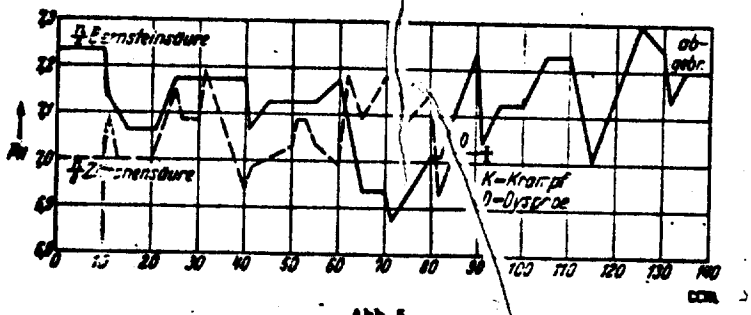

Abb. 5.

12. Citronensäure.

Es wurden n/4 und n/8 Lösungen injiziert. Das p_H der n/4 Säure beträgt 2,15, das der n/8 Säure 2,3. Sie ist wenig lipoidlöslich. Bei oraler

Zufuhr ist sie nach Langecker[1] verbrennbar. Die letale Dosis beträgt für die n/4 Säure 0,28 g bzw. 17,10 ccm pro kg, für die n/8 Säure 0,22 g bzw. 32,90 ccm pro kg Tier. Aus Abb. 5, Kurve 2 ersieht man die Tendenz vorübergehend zu alkalisieren. Das p_H wird vorübergehend erhöht, die Wasserstoffionenkonzentration dementsprechend erniedrigt.

Zusammenfassung.

1. Nach intravenöser Injektion verschiedener Säuren wurde die Änderung der Wasserstoffionenkonzentration im strömenden venösen Blute bestimmt.

2. Man kann drei Grundtypen von Säuren unterscheiden: Solche die das Blut säuern, solche die es alkalisieren und schließlich solche die Ausschläge nach der sauren und alkalischen Richtung geben.

3. Nach ihrem Blutsäuerungsvermögen ergibt sich folgende Reihe: Phosphorsäure > Gluconsäure > Milchsäure > Weinsäure > Salzsäure > Mandelsäure.

4. Nach dem Blutalkalisierungsvermögen geordnet ergibt sich folgende Reihe: Essigsäure > Maleinsäure > Fumarsäure.

5. Sowohl Säuerung als auch Alkalisierung zeigt Äpfelsäure; Bernsteinsäure zeigt nach der sauren, Citronensäure nach der alkalischen Seite vorübergehende Ausschläge.

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[1] Langecker, Hedwig: Naunyn-Schmiedebergs Arch. 171, 744 (1933).

Higgins-'72
Bib No. 168

Fire Technol. 8(2); 120-130. 1972.

Acute Toxicity of Brief Exposures to HF, HCl, NO_2 and HCN with and without CO

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We know that the combustion products of certain materials used inside aircraft include toxic gases. What, however, are the effects of those gases on man after brief exposure, say the time it takes to evacuate an aircraft?

RECENT studies made within the aviation community illustrate the importance of fire, smoke, and fumes in the survivability of, and escape from, aircraft accidents. The Aerospace Industries Association (AIA)[1] examined the importance of fire in 539 fatalities that occurred in 16 impact-survivable accidents and concluded that fatalities in 12 of the accidents were the result of the ensuing fire. Another report[2] showed that, during the period 1955–1964, 12 survivable U.S. air carrier accidents occurred in which 68 crew members and 596 passengers either died by fire or received severe injuries attributable to fire. The same study also showed that 25 crew members and 269 passengers died by fire in other accidents that had some fatalities at impact; the deaths by fire occurred following impact. Snow *et al.*[3] made an exhaustive analysis of the human factors data from three aircraft accidents involving emergency evacuation. All three accidents were impact survivable, but 105 of the 261 passengers died by fire or smoke during attempts to escape the aircraft after impact. Hasbrook *et al.*[4] examined, in some detail, the evacuation pattern of a survivable accident in which 16 passengers died from smoke inhalation and fire.

Comprehensive aircraft safety necessitates the rapid evacuation of passengers immediately following impact-survivable crashes. Many such crashes result in aircraft fires. Since inhalation of the combustion products

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of aircraft components may present a general toxicity hazard, it would be wise to select totally nonflammable materials or, lacking such materials, to select those having the least toxic combustion products.

Marcey and Johnson of the National Aviation Facilities Experimental Center have conducted studies[5] of the burning characteristics of 140 different materials divided into two groups — one group comprised materials now in use in air transport, and the other, materials that have been proposed for future use and that display superior resistance to fire. Also, the Fire Research Section of the National Bureau of Standards conducted research for NAFEC on the smoke and gases produced by burning materials used in the interior of aircraft.[6] Neither test, however, examined the biological effects of the toxic combustion products found.

Of the interior materials presently in use, many formulations contain halogen, cyanide, and nitrogenous moieties, which can react during combustion or pyrolysis to form the corresponding halogen acid gases — hydrogen chloride (HCl) and hydrogen fluoride (HF) — hydrogen cyanide gas (HCN) and nitrogen dioxide (NO_2). Information has been needed concerning the toxicity of these materials when exposure is for a short period of time as might be experienced in the evacuation of a burning aircraft. The present study was conducted to provide this specific information for the gases HCl, HF, HCN, and NO_2. In addition, other experiments were performed to explore the toxic effects of a simultaneous exposure to each of these gases in combination with carbon monoxide (CO).

This study is not an exhaustive evaluation of the toxicity of pyrolysis products. It was designed to establish some criteria for evaluating material in use, or proposed for use, in terms of some of the more commonly produced, easily identifiable toxic gases.

EXPERIMENTAL APPROACH

Ten rats (Wistar) and 15 mice (ICR) per group were exposed to a series of atmospheric concentrations of each test material to determine LC_{50} (Lethal Concentration for 50 percent of the test animals) values. Exposed rats weighed from 250 to 275 g, while mice ranged from 30 to 35 g. Quality control examinations were conducted on each shipment of rats and mice received to assure that healthy animals were used in the toxicity studies.

The animals were exposed in a dynamic flow system using a standard Rochester chamber[7] modified to present the animals with a precisely timed 5-min inhalation challenge. The modification consisted of a cage constructed with gasketed solid ends mounted on a slide track, which was installed in one of the plastic panels on the Rochester chamber. When the desired contaminant concentration was achieved in the chamber, the cage containing animals was rapidly pushed into the chamber and the sealing clamps were secured. After 5 min of exposure, the procedure was reversed, and another experiment was started by readjusting the contaminant concentration.

The animals were observed closely for 7 days following exposure in order to include among the observations any delayed deaths due to pulmonary edema.

A series of animal exposures was conducted to determine the concentration of CO required to produce 25 percent carboxyhemoglobin (COHb) — a level that produces minor CNS effects in man but is not lethal per se. Initial experimental CO concentrations were based on calculations made from the human CO uptake data of Forbes *et al.*[8] Blood carboxyhemoglobin measurements were made on pooled blood samples from simultaneously exposed groups of three or four rats using the method of Goldbaum *et al.*[9] The COHb data for rats are shown in Figure 1. Also shown in this figure is the average COHb value for 24 mice exposed to a single carbon monoxide concentration. Since all animal species have from 0.5 to 1.0 percent endogenous COHb from hemoglobin catabolism, this is essentially a 2-point plot showing that the 25 percent COHb level is achieved in mice during 5 min of exposure to 1500 ppm CO. The 5-min CO concentration required to achieve 25 percent COHb in rats is 2100 pp.

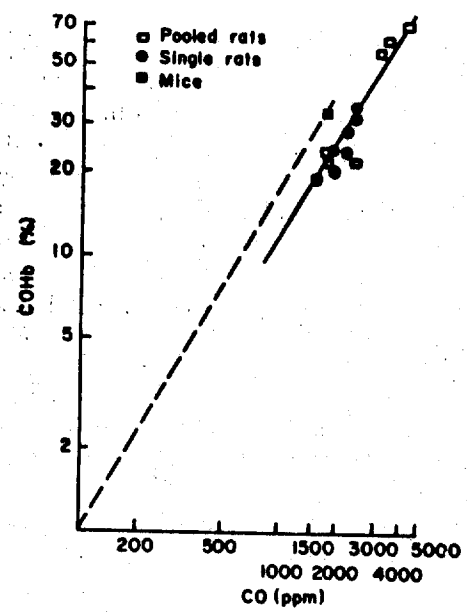

Figure 1. Carboxyhemoglobin formation in rodents exposed to CO for 5 min.

All contaminant exposure concentrations were controlled by continuous monitoring. Hydrogen fluoride, hydrogen chloride, and hydrogen cyanide concentrations were monitored using specific ion electrodes. Air samples were absorbed into a constant flow of aqueous reagent solutions made in accordance with the directions of the electrode manufacturer for pickup of the contaminant and optimum measurement of the ion. Air and solution flow rates were measured precisely for conversion of ion

concentration to airborne contaminant concentration. Calibration curves were prepared by sampling known concentrations of the specific ions made from primary standards. The standards were sodium fluoride (NaF), sodium cyanide (NaCN), and sodium chloride (NaCl).

Nitrogen dioxide was analyzed by continuous spectrophotometric measurement of atmospheric samples absorbed in Saltzman reagent using an AutoAnalyzer.[10] Calibration curves were prepared by analytical measurement of standard gas bag concentrations backed up by standardization with permeation tubes.[11]

The LC_{50} values for each series of exposure to the compounds tested, either singly or in combination with CO, were calculated by the method of Litchfield and Wilcoxon[12] using computer program techniques. This method results in a slope calculated by the method of least squares, which results in the lowest Chi square value possible.

RESULTS AND DISCUSSIONS

Hydrogen fluoride produced pulmonary edema of varying degrees of severity in most of the exposed animals. In animals that died during or shortly after exposure to concentrations above the LC_{50} value, pulmonary hemorrhage was a common finding. Delayed deaths were routinely seen with this compound in exposures below the LC_{50} level, with peak mortality occurring about 24 hours after exposure, although occassional deaths occurred three to four days later. The mortality rates to inhaled HF for rats are presented in Table 1; those rates for mice are in Table 2. The LC_{50} slopes for rats and mice are presented in Figures 2 and 3 respectively. The slopes for the HF and HF + CO exposures are not statistically different. There is no apparent effect attributable to concurrent exposure

Table 1. *Seven-day Mortality Response of Rats Exposed 5 min to HF Gas Singly and in Combination with CO (25% Carboxyhemoglobin)*

HF concentration (*ppm*)	*Deaths* (%) *HF*	*HF + CO*
11,550		0
12,440	10	
12,890		0
15,990		10
17,615	30	
17,750		40
18,580	80	
20,730	70	
21,125		100
22,355		90
22,740		100
23,540		100
25,690	100	
LC_{50}	18,200 ppm	18,208 ppm
95% confidence limits	15,965 to 20,748 ppm	13,698 to 24,202 ppm

TABLE 2. *Seven-day Mortality Response of Mice Exposed 5 min to HF Gas Singly and in Combination with CO (25%) Carboxyhemoglobin)*

HF concentration (*ppm*)	*Deaths* (%) *HF*	*HF + CO*
2,430	0	
4,480		7
4,500	33	
6,220		33
7,410		60
7,615	67	
8,140	53	
8,760		93
10,190		100
11,010	100	
LC_{50}	6,247 ppm	6,670 ppm
95% confidence limits	4,789 to 8,149 ppm	5,690 to 7,807 ppm

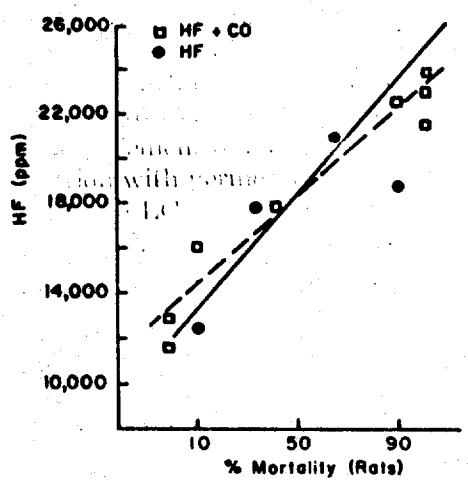

Figure 2. Five-minute LC_{50} for rats exposed to HF singly and in combination with CO.

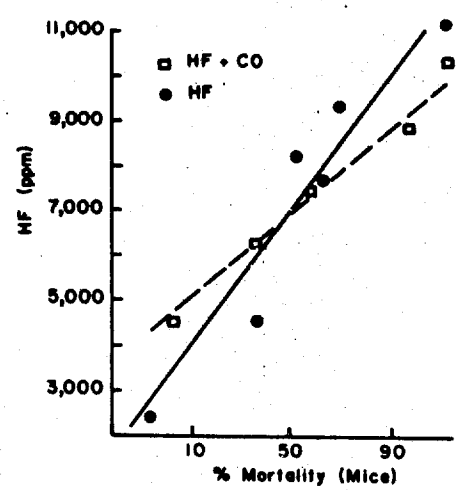

Figure 3. Five-minute LC_{50} for mice exposed to HF singly and in combination with CO.

to carbon monoxide. Indeed, a single slope could be plotted by combining the two sets of exposure data, which would result in a more precise LC_{50} value with narrower 95 percent confidence limits.

Exposure of rats to HCl resulted in LC_{50} values of 40,989 ppm for the pure compound and 39,010 ppm for the combined exposure of HCl and CO, as shown in Table 3. This difference was not statistically significant; neither was the greater difference found in mice as shown in Table 4. The LC_{50} slopes are plotted in Figure 4 for rats and in Figure 5 for mice. Hydrogen chloride is definitely less toxic to rats than is HF; but in mice, the effective range of lethal response to HCl overlaps that of HF. It should be noted that the range of HCl concentrations that had to be used was much wider than that with HF. Consequently, the 5-min toxicity

TABLE 3. *Seven-day Mortality Response of Rats Exposed 5 min to HCl Vapors Singly and in Combination with CO (25% Carboxyhemoglobin)*

HCl concentration (*ppm*)	*Deaths* (%) *HCl*	*HCl + CO*
30,000	0	
32,000	10	
33,980		10
39,850	60	
42,460		80
45,200	70	
49,580		100
56,046		90
57,290	90	
59,280		100
LC_{50}	40,989 ppm	39,010 ppm
95% confidence limits	34,803 to 48,272 ppm	35,049 to 43,419 ppm

TABLE 4. *Seven-day Mortality Response of Mice Exposed 5 min to HCl Vapors Singly and in Combination with CO (25% Carboxyhemoglobin)*

HCl concentration (*ppm*)	*Deaths* (%) *HCl*	*HCl + CO*
3,200	7	
4,920		10
5,060	7	
6,145	13	
6,410	0	
7,525	40	
8,065	13	
9,276	33	
12,805		70
13,655	40	
21,010		80
26,485	87	
27,386		90
30,000	87	
LC_{50}	13,745 ppm	10,663 ppm
95% confidence limits	10,333 to 18,283 ppm	6,921 to 16,428 ppm

of HCl is much less predictable than that of HF. The lowest concentration of HCl causing death in mice was 3200 ppm; however, 100 percent deaths were not achieved at the highest concentration tested (30,000 ppm), which was an order of magnitude greater.

Most NO_2-induced animal deaths resulted from pulmonary edema and were seen within 12 hours after exposure; a few animals exhibited pulmonary hemorrhage. Again, as shown in Tables 5 and 6, there was no significant difference between exposure to NO_2 alone and exposure to NO_2 in combination with CO (Figures 6 and 7). Simultaneous exposure of rats to CO and NO_2 resulted in a slightly higher LC_{50}, an indication of

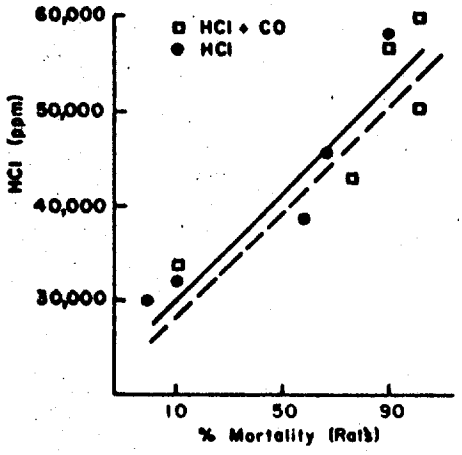

Figure 4. Five-minute LC_{50} for rats exposed to HCl singly and in combination with CO.

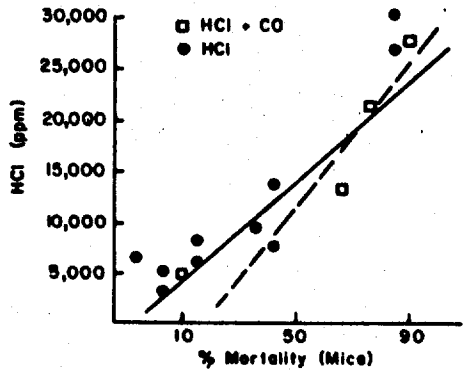

Figure 5. Five-minute LC_{50} for mice exposed to HCl singly and in combination with CO.

TABLE 5. *Seven-day Mortality Response of Rats Exposed 5 min to NO_2 Singly and in Combination with CO (25% Carboxyhemoglobin)*

NO_2 concentration (ppm)	Deaths (%) NO_2	Deaths (%) NO_2 + CO
260	10	
270		0
550	40	
580		0
590	30	
750		20
840	30	
850	30	
1,000		40
1,200	50	
1,250	90	
1,380	100	
1,660		80
LC_{50}	831 ppm	1,140 ppm
95% confidence limits	566 to 1,240 ppm	720 to 1,707 ppm

a slightly less toxic response. The results of these LC_{50} studies of NO_2 on rats are almost identical to those reported by Gray *et al.*[12]; the latter found a 5-min LC_{50} value of 832 ppm, which compares well with our value of 831 ppm.

In HCN exposures, either singly or in combination with CO, all deaths occurred during the exposure period or within 20 min after exposure. There were no delayed deaths. The results of the experiments are given in Tables 7 and 8 for rats and mice, respectively. Carbon monoxide at the 25 percent carboxyhemoglobin level had no effect on the acute toxicity of HCN. Because the primary effect of HCN intoxication is the blocking

TABLE 6. *Seven-day Mortality Response of Mice Exposed 5 min to NO_2 Singly and in Combination with CO (25% Carboxyhemoglobin)*

NO_2 concentration (ppm)	Deaths (%) NO_2	Deaths (%) NO_2 + CO
260	7	
550	27	
580		0
590	0	
840	7	
850	0	
950		7
1,200	40	
1,250	13	
1,380	47	
1,500		53
1,990	20	
2,280		74
2,560	67	
2,950	74	
2,980	100	
3,280		93
LC_{50}	1,880 ppm	1,644 ppm
95% confidence limits	1,354 to 2,626 ppm	1,203 to 2,247 ppm

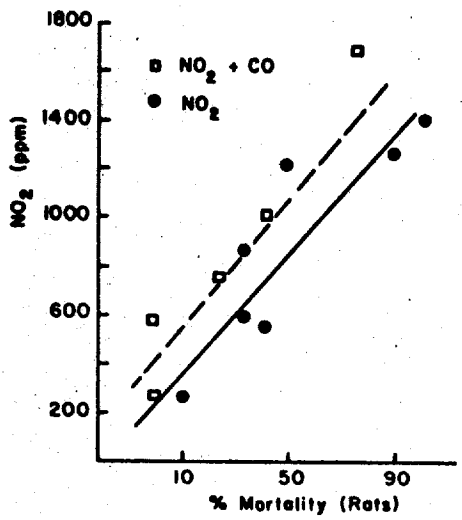

Figure 6. Five-minute LC_{50} for rats exposed to NO_2 singly and in combination with CO.

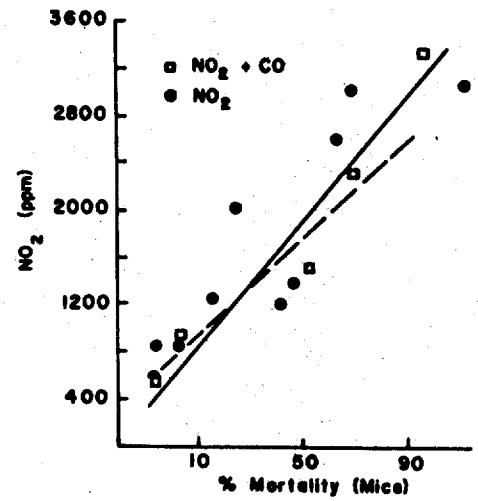

Figure 7. Five-minute LC_{50} for mice exposed to NO_2 singly and in combination with CO.

of intracellular oxygen transport through the cytochrome system, the slightly decreased extra-cellular oxygen transport was not an important factor. In aircraft fires, it is possible that the buildup of CO will be faster than the buildup of other toxic gases. In order to determine the effect of prior exposure to CO on mortality rates of HCN, two separate groups of rats were exposed to HCN immediately following exposure to CO. The

TABLE 7. *Seven-day Mortality Response of Rats Exposed 5 min to HCN Singly and in Combination with CO (25% Carboxyhemoglobin)*

HCN concentration (ppm)	*Deaths (%)*	
	HCN	*HCN + CO*
280		0
283	0	
334		10
357	10	
368	20	
497	20	
504		70
557		70
583	80	
690	100	
LC_{50}	503 ppm	467 ppm
95% confidence limits	403 to 626 ppm	395 to 553 ppm

TABLE 8. *Seven-day Mortality Response of Mice Exposed 5 min to HCN Singly and in Combination with CO (25% Carboxyhemoglobin)*

HCN concentration (ppm)	*Deaths (%)*	
	HCN	*HCN + CO*
188		0
200	0	
283	27	
288		61
300		40
319		87
357	80	
360		87
368	67	
414	80	
427	100	
LC_{50}	323 ppm	289 ppm
95% confidence limits	276 to 377 ppm	245 to 340 ppm

exposure to CO resulted in 25 percent of COHb for one group of rats and 50 percent COHb for the second group. The results of these exposures were no different from those in which CO and HCN were used simultaneously. The data are presented graphically in Figure 8 for rats and in Figure 9 for mice.

The CO exposure superimposed on the individual containmant exposures might have resulted in more rapid responses, i.e., a decrease time to death. Such a response would be important in consideration of potential hazards for aircraft passengers. We compared the mortality time data of single and combined exposures and found no significant differences or trends.

These acute studies show that the toxicity ranking of the four materials tested is HCN, NO_2, HF, and HCl, in decreasing order. However, the

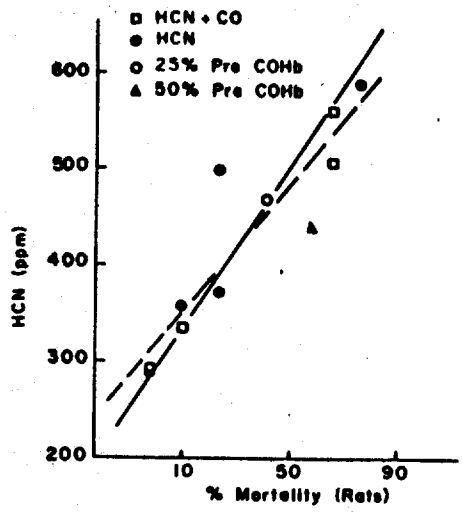

Figure 8. Five-minute LC_{50} for rats exposed to HCN singly and in combination with CO.

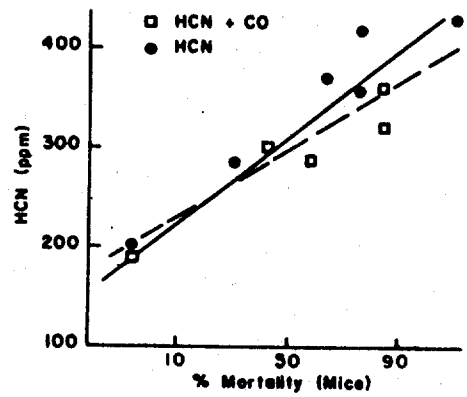

Figure 9. Five-minute LC_{50} for mice exposed to HCN singly and in combination with CO.

hazard rating of various plastic formulations would require experimental determination of the amounts of these materials produced by pyrolysis of comparable quantities (i.e., although the combustion of 100 pounds of a cyanide containing plastic might produce a concentration of 200 ppm in an aircraft cabin, it is possible that the combustion of some chloride-producing plastic might yield cabin concentrations as high as 50,000 ppm; in this event, the chloride-containing plastic would present the greater hazard).

We have shown in these experiments that carbon monoxide concentrations that are not hazardous to life do not enhance the toxic response to the four substances tested. Some LC_{50} values for CO combined with another toxic compound are slightly lower than those of the compounds alone. However, the series of exposures of rats to NO_2 with 25 percent carboxyhemoglobin established prior to exposure indicates that there is neither a trend toward enhancement of toxicity nor a protective value, but that the results vary randomly to the extent that one could expect from repeated series of 5-min LC_{50} values for the various compounds.

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Note: This paper is an adaptation of Federal Aviation Administration Report FAA-AM-71-41 entitled "The Acute Toxicity of Brief Exposures to HF, HCl, NO_2 and HCN Singly and in Combination with CO."

Hunt-'72
Bib No. 179

Jour. Physiol. 222(1):187-208.

. Physiol. (1972), 222, pp. 187–208
'ith 5 text-figures
'rinted in Great Britain

THE SLOWING OF GASTRIC EMPTYING BY FOUR STRONG ACIDS AND THREE WEAK ACIDS

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(*Received 21 October 1971*)

SUMMARY

1. Test meals of 750 ml. water containing a range of concentrations of hydrochloric, nitric, sulphuric, cyclamic (cyclohexylsulphamic), tartaric, L-ascorbic and citric acids were instilled into the stomachs of sixteen healthy subjects.

2. The greater the concentration of an acid in the instilled meal, the greater was the volume of meal recovered after a fixed interval.

3. For the weak acids, tartaric, ascorbic and citric, the concentrations which gave a recovery of 450 ml., out of the 750 ml. instilled, increased with increase in the molecular weight of the acid.

4. For the strong acids, hydrochloric, nitric, sulphuric and cyclamic, the concentrations which gave a recovery of 450 ml., out of the 750 ml. instilled, were approximately equal. These concentrations were less than those for weak acids.

5. The strong acids with high molecular weights were slightly more effective in slowing gastric emptying than were those with low molecular weights.

6. The approximate equality of effectiveness of nitric, sulphuric and cyclamic acids to hydrochloric acid is attributed to the presence of chloride ion in the duodenal contents. Thus all the strong acids instilled produce an environment of hydrochloric acid around the receptor.

7. A model for a duodenal receptor responding to acids is proposed.

INTRODUCTION

It has been known since the beginning of this century that hydrochloric acid added to test meals slows gastric emptying (Hedblom & Cannon, 1909). This effect is believed to be mediated by duodenal receptors which are stimulated by the test meal as it leaves the stomach (Shay & Gershon-Cohen, 1934). These receptors inhibit the peristaltic activity of the stomach

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(Thomas, Crider & Mogan, 1934), and possibly increase the resistance of the duodenum to filling (Weisbrodt, Wiley, Overholt & Bass, 1969).

The greater the concentration of hydrochloric acid in a test meal the greater is the slowing of gastric emptying. This finding is consistent with the notion that the pH or the concentration of hydrogen ions in the gastric effluent determines the degree of stimulation of the duodenal receptors (Hunt & Knox, 1969). Hunt & Knox (1969) also found that for a given concentration, acids with high molecular weights were less effective in slowing gastric emptying than were those with low molecular weights. Of the nine acids they tested, all except hydrochloric acid were weak acids with pK values greater than 2·1. However, the results with hydrochloric acid were consistent with those for weak acids. For example, acetic acid (mol. wt. 60), which is very weak, was almost as effective as hydrochloric acid (mol. wt. 36·5), in spite of the hydrogen ion activity being at least 100 times greater with hydrochloric acid than with acetic acid. This finding forced Hunt & Knox (1969) to discard the notion that the duodenal receptors were responding simply to the pH of the test meal as it left the stomach. From previous work (Hunt & Pathak, 1960) it was apparent that the slowing effect of most salts on gastric emptying was of a minor order compared with that of acids even though the anions were common. Thus the duodenal receptors studied by Hunt & Knox (1969) were truly responding to acid. Their comparision of eight weak acids with hydrochloric acid was not entirely satisfactory since the molecular weight of the hydrochloric acid was less than those of all the other acids. What was needed was a set of strong acids with a range of molecular weights spanning that of the weak acids. Fortunately there are three such strong acids which can be given to man, nitric acid (mol. wt. 63) sulphuric acid (mol. wt. 98) and cyclamic acid (mol. wt. 179). In order that weak and strong acids should be studied in the same group of subjects, tests on the previously studied tartaric and citric acids were repeated. One new weak acid, ascorbic, was included in the tests.

The present study is a comparison of the action of weak and strong acids on the receptors slowing gastric emptying.

METHODS

The methods used were the same as those described by Hunt & Knox (1969). Briefly, 750 ml. test meals, containing various concentrations of different acids and phenol red as a marker, were instilled into fasting subjects after their stomachs had been washed out with 250 ml. tap water.

After an interval, fixed for each subject, but varying between 10 and 25 min for different subjects, the gastric contents were withdrawn and the stomach washed out again. From the amount of phenol red recovered, the volume of original meal

recovered was computed. Phenol red is not adsorbed or absorbed by the stomach of man (Ivey & Schedl, 1970) even at pH 1·0.

Statistical methods

Linear regression equations for volume of original meal recovered against concentration of acid in the given meal were computed after checking by eye that a straight line adequately represented the results.

Because there is some uncertainty about the threshold concentration of acid required to operate the duodenal receptors, the regression lines were fitted only for concentrations of 10 mN upwards. This matter is discussed at length in a previous paper (Hunt & Knox, 1969).

The concentrations of acid required to give a recovery of 400 ml. (Table 2) or 450 ml. (Table 4) were determined from the regression equations with concentration as dependent variable. To be consistent with the earlier paper (Hunt & Knox, 1969) the constants of the alternate regression, with volume as dependent variable, are given in Tables 1 and 3.

RESULTS

Hydrochloric acid and sulphuric acid

Fig. 1 shows the volumes of 750 ml. meals recovered plotted against the concentrations of sulphuric acid in the relevant meals for subject C.L. This subject showed the usual increase in volume of meal recovered as the concentration of acid in the instilled meal was increased. The figure also indicates how the concentration giving a recovery of 450 ml., in this instance 28 m-equiv/l., was determined.

Table 1 shows the regression constants (volume as dependent variable) for the relation between volume of meal recovered after a fixed time interval and the concentration of hydrochloric acid or sulphuric acid in the instilled meal.

To compare the effectiveness of the two acids in slowing gastric emptying, the concentrations giving recoveries of 400 ml. were computed from the regression constants with concentration as dependent variable. The concentration giving a recovery of 400 ml. was chosen because this was the largest volume of recovery common to all subjects. Table 2 shows that the mean concentration of hydrochloric acid giving a recovery of 400 ml. was 25 mM, and of sulphuric acid 23 mN.

If the effectiveness of all acids were a function of their molecular weight it would be expected from Fig. 5 (Hunt & Knox, 1969) that about 35 mN sulphuric acid (mol. wt. 98) would have the same effect as 25 mN hydrochloric acid (mol. wt. 36·5). This difference of 10 mN should have been readily detected in the present experiments, since the standard error of the mean within-subject difference between the concentrations of hydrochloric and sulphuric acids giving recoveries of 400 ml. was only ± 1·55 mN. Thus sulphuric acid is anomalous since molecular weight does not determine its effectiveness.

TABLE 1. The effect of acid in test meals on the volume of meal recovered from the stomach after a fixed time interval. Relation between concentration (x mN) of hydrochloric acid and of sulphuric acid in test meals and the volume of meal (y ml.) recovered after a fixed time interval. $y = a + bx$ so that 'a' is the volume of meal computed to be recovered with zero concentration of acid. The mean volume of water recovered was determined by experiment

Subject...	K.G.	R.D.	C.L.	M.A.	B.L.	W.S.	B.N.	R.B.	C.T.	M.E.
Duration of meals (min)...	25	20	20	15	20	20	25	20	25	20
Hydrochloric										
a	36	131	181	212	108	212	175	75	314	60
b	8·60	8·74	8·33	11·30	9·00	7·46	9·53	15·48	2·57	10·76
S.E. of slope	2·08	1·91	2·14	2·31	2·03	3·71	2·49	4·23	2·44	0·58
n	17	20	22	16	15	8	14	14	12	3
Range of concentrations	10–30	10–30	10–30	10–30	10–30	10–30	10–30	10–30	10–31	10–30
Sulphuric										
a	85	180	66	194	169	310	301	223	252	210
b	7·69	5·97	13·05	5·98	5·54	3·29	4·62	5·04	5·69	6·02
S.E. of slope	2·19	1·72	1·45	1·96	3·08	2·98	2·71	3·92	2·84	3·43
n	20	19	21	18	12	9	7	11	9	9
Range	10–33	10–33	10–33	10–33	10–26	10–33	10–33	10–33	10–33	10–33
Mean volume of water remaining	110·5	158·3	190·6	163·6	240·5	330·0	308·5	224·4	386·2	240·0
S.E. of mean	19·8	23·5	18·6	25·7	64·0	33·6	24·1	41·1	19·6	23·3
n	6	9	13	9	4	4	4	5	4	9

TABLE 2. Concentrations (mN) of hydrochloric and sulphuric acids giving recoveries of 400 ml. after a fixed time interval. The results were calculated by substituting 400 ml. in the regression equation $x' = a' + by'$, where x' = concentration of acid in meal, and y' = volume of meal recovered

Subject	Hydrochloric (mN)	Sulphuric (mN)
K.G.	31·1	27·9
R.D.	25·7	26·6
C.L.	22·6	24·0
M.A.	24·2	24·8
B.L.	27·4	22·8
W.S.	21·8	19·5
B.N.	23.8	19·2
R.B.	19·9	22·2
C.T.	22·1	19·8
M.E.	31·5	21·6
Mean	25·0	22·8
S.E.	± 1·24	± 0·24

Within-subject S.E. of difference ± 1·55 mN.

Hydrochloric acid, nitric acid and cyclamic acid

Table 3 shows the relation (volume as dependent variable) between the concentrations of acids (mN) and the volumes of meal recovered for hydrochloric (mol. wt. 36·5), nitric (mol. wt. 63), sulphuric (mol. wt. 98) and cyclamic (mol. wt 179) acids. All are strong acids, the least strong being cyclamic (cyclohexylsulphamic) with a pK of less than 0·9.

Table 4 shows the concentrations of these acids computed (concentration as dependent variable) to give recoveries of 450 ml. The means for hydrochloric, nitric, sulphuric and cyclamic acids are 35·2, 34·8, 29·8 and 24·7 mN respectively. Thus to a first approximation all four strong acids are about equally effective in slowing gastric emptying. There is certainly no decrease in effectiveness with molecular weight as there is with weak acids.

Amongst these strong acids there is a tendency for those with the higher molecular weights, sulphuric and cyclamic, to be slightly more effective than hydrochloric acid and nitric acid. To put this on a quantitative basis a regression equation was computed relating molecular weight to the concentration of acid giving a recovery of 450 ml. To eliminate the considerable between-subject variation, the concentration of each acid giving a recovery of 450 ml. was expressed as a percentage of the mean concentration of the four acids giving 450 ml. recoveries for that subject. The regression was found to be

$$x = 119 - 0{\cdot}197y, \quad \text{S.E. of slope} = \pm 0{\cdot}063, \quad P = 0{\cdot}005,$$

where

x = concentration of acid giving 450 ml. recovery, as % mean for four acids in each subject:

y = molecular weight of the strong acid.

The significant negative slope in the regression for these results indicates that with increased molecular weight these four strong acids became more effective in slowing gastric emptying.

TABLE 3. The effect of acid in test meals on the volume of meal recovered from the stomach after a fixed time interval. Relation between concentrations (x mN) of acid in test meals and the volume of meal (y ml.) recovered after a fixed time interval. $y = a + bx$ so that 'a' is the volume of meal computed to be recovered with zero concentration of acid. The mean volume of water recovered was determined by experiment

Subject	H.T.	B.G.	W.Y.	F.S.	W.D.	E.S.	E.E.
Duration of meals (min) ...	20	20	20	20	20	20	20
Hydrochloric							
a	82	30	184	280	49	105	499
b	6·37	10·90	6·63	5·92	9·72	9·13	7·81
S.E. of slope	1·71	4·25	1·63	2·36	1·92	2·87	17·59
n	10	6	12	10	11	8	5
Range	23–50	29–40	18–40	8–41	8–40	8–40	14–23
Cyclamic							
a	118	−8·5	258	107	215	94	185
b	8·20	17·27	5·98	16·14	8·08	11·81	11·73
S.E. of slope	1·16	4·81	1·97	8·10	5·57	3·44	1·50
n	12	7	8	9	13	10	12
Range	10–67	3–25	10–28	10–26	8–30	10–30	0–30
Citric							
a	—	−24	263	293	−55	329	202
b	—	3·84	1·55	1·77	4·03	1·52	3·20
S.E. of slope	—	0·71	0·79	0·40	1·11	0·84	0·59
n	—	4	6	8	6	5	5
Range	—	51–151	51–151	45–151	51–127	45–151	45–127
Ascorbic							
a	162	184	326	223	96	308	—
b	1·81	2·86	1·82	3·00	2·80	1·95	—
S.E. of slope	0·44	0·97	0·59	0·56	0·56	1·43	—
n	18	7	9	12	13	7	—
Range	10–155	28–105	33–119	12–125	13–137	18–104	—

TABLE 3 (*cont.*)

Subject	H.T.	B.G.	W.Y.	F.S.	W.D.	E.S.	E.E.
Duration of meals (min)...	20	20	20	20	20	20	20
Tartaric							
a	—	155	164	183	177	161	—
b	—	3·56	3·95	4·33	3·22	4·43	—
S.E. of slope	—	1·50	2·43	1·46	1·15	2·14	—
n	—	7	5	5	6	6	—
Range	—	26–86	38–73	38–73	38–88	26–73	—
Nitric							
a	95	90	—	152	—	203	—
b	7·65	6·39	—	7·43	—	4·44	—
S.E. of slope	1·27	3·40	—	1·87	—	1·58	—
n	13	12	—	10	—	13	—
Range	5–36	6–39	—	5–36	—	1–29	—
Sulphuric							
a	109	69	176	462	226	147	499
b	6·89	7·67	6·42	4·51	4·67	7·80	7·82
S.E. of slope	2·46	2·18	2·62	8·76	2·77	4·91	17·6
n	10	6	13	10	12	7	5
Range	10–35	20–62	14–38	17–29	10–38	17–38	14–23
Mean volume of water remaining	145	147	192	211	173	204	180
S.E. of mean	—	25·5	19·3	20·2	30·3	36·6	11·0
n	—	8	6	10	13	8	3

Tartaric, ascorbic and citric acids

Table 3 shows the regression constants (volume as dependent variable) describing the relations between the concentrations of tartaric, ascorbic and citric acids and the volumes of the meals recovered.

From these regression equations the concentrations of tartaric acid (mol. wt. 150) and citric acid (mol. wt. 192) giving recoveries of 450 ml. were computed for each subject. For each subject these two concentrations were plotted against the molecular weights of the respective acids. From a straight line connecting these two points the concentration of L-ascorbic acid (mol. wt. 176) expected to give a recovery of 450 ml. was read off. The mean of the values expected for ascorbic acid was 92 mN; the mean of the observed values was 83 mN (within-subject S.E. ± 7·6). The difference between the expected and observed values is not statistically significant.

TABLE 4. Concentrations of acid (mN) in test meals giving recoveries of 450 ml. after a fixed time interval. n = number of observations included in the mean. The concentrations were calculated by substituting 450 ml. in the regression equation $x' = a' + by'$ where x' = concentration of acid in meal and y' = volume of meal recovered. The value in parentheses for E.E. was computed when the results for meals of water were included in the regression. For all other subjects the results for the meals of water were omitted. The three values for n are given to allow comparisons to be made. * Computed from Table 1 (Hunt & Knox, 1969)

Subject	Ascorbic	Citric	Cyclamic	Hydrochloric	Sulphuric	Tartaric	Nitric
B.G.	83·9	122·1	19·2	37·4	45·7	72·3	31·5
H.T.	116·1	114·0*	38·1	49·1	35·1	68·7*	40·9
F.S.	71·4	90·4	19·4	28·5	21·7	60·1	33·8
E.S.	50·9	89·0	26·7	34·6	29·5	59·1	32·9
W.Y.	68·1	112·4	27·9	36·5	30·4	63·5	—
W.D.	105·3	117·6	20·1	38·2	27·8	77·0	—
E.E.	—	78·0	(21·6)	21·9	18·8	—	—
			30·9				
Mean n 7	—	103·4	24·7	35·2	29·8	—	—
n 6	82·5	107·6	25·2	37·4	31·7	66·8	—
n 4	—	—	25·9	37·4	33·0	—	34·8

Thus the results for ascorbic acid fit into the pattern found for the other weak acids.

DISCUSSION

Figs. 1 and 2 summarize our information about the action of different acids in slowing gastric emptying. From Fig. 1 it may be seen that on increasing the concentration of sulphuric acid in the given meal from 10 to 35 mN the recovery of the meal after a fixed interval increased from about 200 up to 500 ml. A similar effect, to different degrees, was seen with all thirteen acids examined.

The relationship between the effectiveness of acids in slowing gastric emptying and their molecular weights is shown in Fig. 2. The ordinate shows the mean concentration (m-equiv/l.) of an acid giving a recovery of 450 ml. after a fixed interval following a 750 ml. meal containing the relevant acid. The higher the value on the ordinate, the less effective was the acid in slowing gastric emptying. It may be seen that the concentrations giving recoveries of 450 ml. increase with molecular weight for acetic, propionic, lactic, butyric, hexanoic, tartaric, ascorbic and citric acids. All these acids are more or less weak, the lowest pK being that for tartaric, 3·0. Molecular weight thus seems to be the main determinant of effectiveness of weak acids in slowing gastric emptying. The relationship is stronger than appears in Fig. 2 since this includes all our results in several different

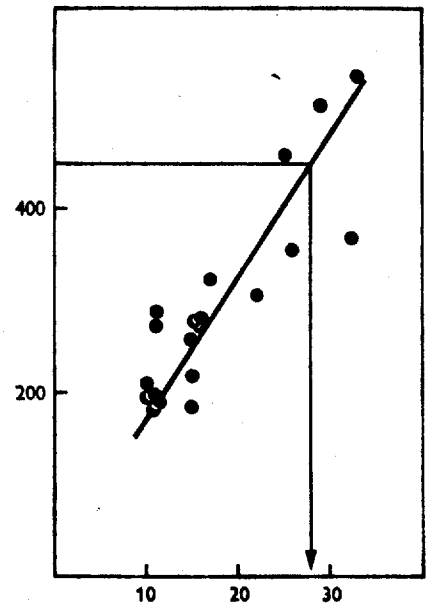

Fig. 1. The effect of increasing the concentration of sulphuric acid in instilled test meals on the volume of meal recovered after 20 min. Ordinate: volume of meal recovered at 20 min (ml.). Abscissa: concentration of acid (mN).

groups of subjects. In a previous paper (Hunt & Knox, 1969) the corresponding correlation coefficient for six acids compared in fourteen subjects was 0·997.

Fig. 2 shows that the results for three strong acids, nitric, sulphuric and cyclamic, stand apart from those for weak acids with similar molecular weights. In spite of their higher molecular weights these three strong acids are at least as effective as hydrochloric acid.

Is acid the stimulus to the receptor?

That acetic acid, a weak acid (pK 4·8), and hydrochloric acid, a very strong acid, should be nearly equal in stimulating a receptor allegedly responding to acid, is remarkable. That in addition the molecular weights of acids, which is entirely a property of their anions, should dominate the responses of the receptor to different acids must raise the question as to whether or not the receptor is truly responding to acid. In concentrations up to 125 mN, solutions of sodium chloride or sodium citrate do not slow gastric emptying, in fact they leave the stomach about twice as quickly as

water (Hunt & Pathak, 1960; Hunt & Knox, 1962). Moreover, there are results for calcium, potassium and ammonium salts which show that most anions have only relatively weak actions on gastric emptying (Hunt & Pathak, 1960). There seems to be no escape from the conclusion that the receptor studied in the present work responds to acid.

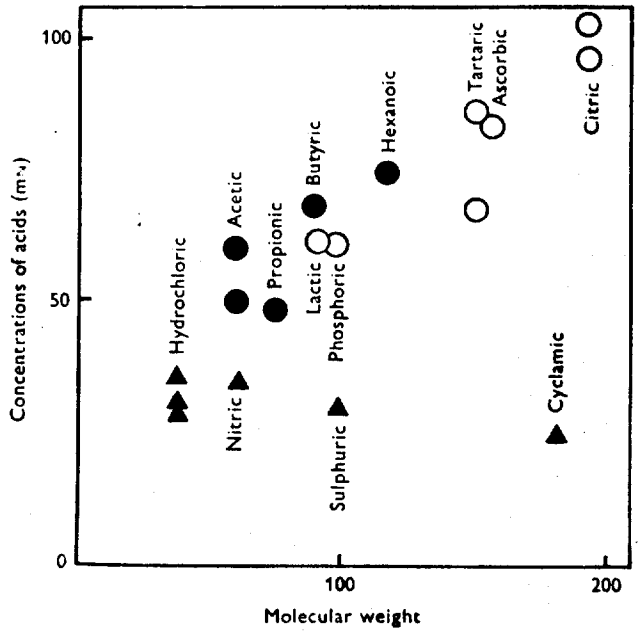

Fig. 2. The relation between molecular weights of weak and strong acids and their concentration in 750 ml. test meals which gave recoveries of 450 ml. from the stomach after a fixed interval. For hydrochloric, acetic, tartaric and citric acids there is more than one point because the experiments were replicated in different groups of subjects. Ordinate: concentration of acids (mN). Abscissa: molecular weight.
Strong acids (▲): hydrochloric, nitric, sulphuric, cyclamic. *Fatty acids* (●): acetic, propionic, butyric, hexanoic. *Other acids* (○): lactic, phosphoric, tartaric, ascorbic, citric.

The receptive process as analogous to titration

It would be easy to envisage a duodenal receptor responding to acid much like a glass electrode. A potential across a cell membrane varying with the concentration gradient for hydrogen ion (strictly H_3O^+), could serve as a generator potential for a neural mechanism. However, such a simple receptor could not give almost equal graded responses proportional to the concentration of hydrochloric acid and proportional to the concentration of acetic acid. A change from 10 to 100 mN with hydro-

chloric acid would give nearly a tenfold increase in hydrogen ion activity from pH 2·0 to 1·1. But for acetic acid such a change in concentration would give only a threefold increase in hydrogen ion activity, from pH 3·5 to 3·0, because acetic acid dissociates feebly. Yet the receptor responses to these two acids have the ratio 1·6 to 1, which is accounted for by the ratio of their molecular weights (1·6 to 1).

Given solutions of a variety of acids with different pK values the simplest way to determine their concentrations in m-equiv/l. is to titrate aliquots and the model proposed for the duodenal receptor responding to acids is assumed to work by a process analogous to titration.

The end-point of the titration process

Fig. 2 shows on the ordinate the mean concentration of each of thirteen acids which gave recovery of 450 ml. after a fixed interval.

These concentrations were all measured by titrating the acids to pH 6·5. Titrating to any substantially different pH would give neither a rectilinear nor an orderly relationship between concentrations which gave recoveries of 450 ml. and molecular weights. For example, it makes little difference to the recorded concentration of hydrochloric acid whether it is titrated to pH 3, pH 4, etc. However, for acetic acid, at pH 3 the titration of its proton donors has scarcely begun and even at pH 5·8 the titration is not complete, the pK of acetic acid being 4·8.

The decision to titrate the acids in the test meals to pH 6·5 was taken during experiments with citric acid and its sodium salts because it resulted in apparent equal deliveries to acid to the duodenum (Hunt & Knox, 1962). The same end-point was used for the titration of other acids studied by Hunt & Knox (1969). As pointed out above, it makes little difference with the majority of acids used in these experiments whether they are titrated to pH 6 or pH 8 since the titration is virtually complete by pH 6. On the other hand, phosphoric acid has three pKs (2·1, 7·2, 12·7), so that changes in the range pH 6–8 cause the dissociation of acid groups and measurable quantities of base have to be added to bring about this dissociation. It thus makes a considerable difference in determining the concentration of phosphoric acid whether it is titrated to pH 6 or to pH 8. By experiment we found that the point for phosphoric acid (mol. wt. 98) in Fig. 2 fell on the line for the weak acids when the phosphoric acid was titrated to pH 6·5, at which pH about 45% of the proton donors have dissociated. This amounts to saying that the point for phosphoric acid in Fig. 2 can be manipulated on to the line for the other weak acids by specifying that the end-point for all the titrations is pH 6·5. We therefore conclude that our model should be specified as titrating to pH 6·5 to account for the results in Fig. 2.

The lack of effect of oil/water partition coefficient on the effectiveness of acids

It seems possible that acids might stimulate a receptor by penetrating a cell membrane and then increasing the activity of an enzyme by the fall in intracellular pH. Since cell membranes are thought of as being lipoprotein, acids soluble in such membranes would be expected to penetrate the membrane readily. If this were so, fat soluble acids, especially weak acids in the uncharged state, would be more effective than acids insoluble in fat. The finding that the points for acetic, propionic, butyric and hexanoic acids, with benzene/water partition coefficients varying from 0·02 to 38 (Brown & Bury, 1923), fall equally about the line in Fig. 2 for more polar acids indicates that fat solubility does not confer increased effectiveness on an acid. Our current interpretation is that the acids can affect the receptor element without themselves diffusing through a lipid membrane.

The lack of effect of the number of replaceable hydrogen ions in acids

In Fig. 2 the values on the ordinate are plotted in m-equiv/l. If the concentrations which gave recoveries of 450 ml. were specified in m-mole/l. the Figure would lose its orderliness. The value on the ordinate gives twice the weight to an acid with two replaceable hydrogen ions as that which it gives to an acid with one. This is a further point consistent with the notion that the receptor responds to acid, not to anions. Nevertheless, it is the molecular weight of its anion which determines the effectiveness of an acid after the inequality of the acids has been eliminated by recording their concentrations in m-equiv/l.

For weak acids, and the majority of the results in Fig. 2 are for weak acids, it is presumably mainly molecules of undissociated acid which move towards the receptor. However, arriving at the receptor, lactic acid gives rise to one hydrogen ion while citric acid gives rise to three hydrogen ions. Expressing concentrations as m-equiv/l. eliminates the distinction between acids with different numbers of replaceable protons.

Findings consistent with diffusion to the titration site

In man. Fig. 2 shows that the higher their molecular weight the less effective are weak acids in slowing gastric emptying, which is consistent with the effect of an acid being limited by its rate of diffusion to a hypothetical titration site.

Since Fig. 2 relates to concentrations of different acids giving equal slowing, it will be assumed that equal fluxes of different acids to the titration site are given by the equi-active concentrations on the ordinate. It is worth remarking that these different concentrations (C_{eq}) are measured at a time when the intragastric volumes and the volumes that have passed

into the duodenum (750–450 ml.) are the same for each acid. Assuming, for reasons given above that the concentration of acid at the titration site is zero, the steady state flux to the site is given by

$$J_{450} = DC_{450}\,A/X, \tag{1}$$

where

J_{450} = flux giving recovery of 450 ml., assumed equal for all acids,
C_{450} = concentration of an acid giving a recovery of 450 ml.,
D = diffusion coefficient for the acid,
A = area for diffusion, and
X = length of diffusion channel.

Since A/X is assumed constant within an individual, it does not influence ratios of fluxes and may be eliminated.

As we have no precise information about the concentration of acid at the duodenal end of the diffusion channels we assume that it is linearly related to the concentration, C_{450}, in the given meal when the volume recovered is 450 ml.

There are apparently very few diffusion coefficients for acids available in the literature. We have assumed that the relation between diffusion coefficient, D, and mol. wt. (MW), following Graham's Law has the form

$$D = y\,\frac{1}{MWp} \quad \text{where } y \text{ and } p \text{ are constants.} \tag{2}$$

Substituting in (1) for D and rearranging we obtain

$$\frac{1}{C_{450}} = \frac{y}{J_{450}}\cdot\frac{1}{MWP}.$$

Taking logarithms gives a relationship with the form

$$\log\frac{1}{C_{450}} = \log\frac{y}{J_{450}} + p\log\frac{1}{MW},$$

where y and J_{450} are constants and y/J_{450} is a constant, so that a value for p could be conveniently obtained by plotting log $10^3/C_{450}$ against log $10^3/MW$ using all our results for hydrochloric acid and the seven weak acids which we have studied. The results for phosphoric acid were excluded because the concentration giving 450 ml. was made to fit the other results in the interest of determining the pH to which the receptor appears to titrate. The results for propionic acid were excluded because it was anomalous in our studies both as an acid and as a salt (Hunt & Knox, 1968). The value for p, 0·49, obtained as the slope of the log log plot was then used to construct Fig. 3, the arithmetic plot of

$$\frac{10^3}{C_{450}} \quad \text{against} \quad \frac{y}{J_{450}}\cdot\frac{10^3}{MW^{0\cdot49}}.$$

The fairly close fit of our experimental points to the line ($r = 0{\cdot}97$) is consistent with C_{450} being determined by the coefficient of diffusion calculated on the basis of eqn. (2).

The use of C_{450} for the comparison of the effectiveness of acids is open to the objection that the choice of recoveries of 450 ml. is arbitrary. We have therefore made calculations corresponding to those leading up to Fig. 3, but using the slopes (S) of the lines relating the concentrations of acids to recoveries of meal, as in Fig. 1, against $1/MWP$, giving a value for P of 0·65. Fig. 4 shows the fit ($r = 0{\cdot}95$) of the mean values for S for all our eight results plotted against $1/MW^{0{\cdot}65}$. These results and two other values of p

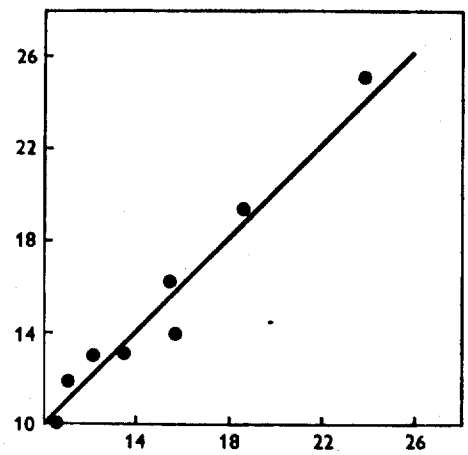

Fig. 3. Relation between mean concentrations of hydrochloric acid and seven weak acids giving recoveries of 450 ml. and their molecular weight (MW). Ordinate: 10^3/mean concentrations of acids giving recoveries of 450 ml. Abscissa: 4·7 ($10^3/MW^{0{\cdot}48}$).

obtained from plots of diffusion coefficients, taken from the literature, against $1/MWP$ have been collected in Table 5. It may be seen that all four values of p so obtained are similar, which is consistent with the notion that diffusion is a rate-limiting step in our system.

Our mean values for acetic and citric acid for C_{450} and S, the slope of Fig. 1, are based on some hundreds of test meals in twenty subjects for each acid. For these acids there are published diffusion coefficients. The diffusion hypothesis requires that the product $C_{450} \times$ diffusion coefficient and the quotient

$$\frac{S \text{ (the slope of Fig. 1)}}{\text{diffusion coefficient}}$$

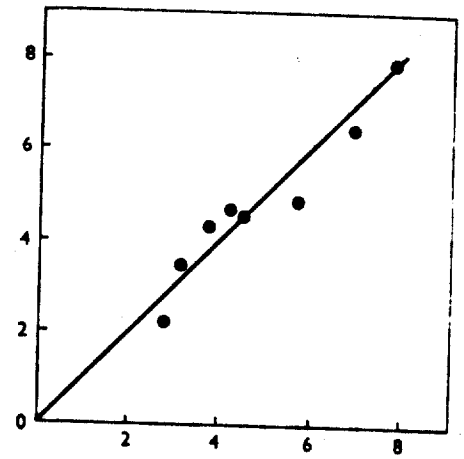

Fig. 4. Relation between mean slopes of volume recovered on concentrations of acids in the meals for hydrochloric acid and seven weak acids. Ordinate: slope of volume recovered on concentration of acid in meal (increase in volume recovered/m-equiv. l. increase in concentration of acid in meal). Abscissa: 0·206 ($10^4/MW^{0.45}$).

TABLE 5. A comparison of the power function (p) of mol. wt. (MW) obtained by assuming that diffusion coefficients (D) and indices of effectiveness of acids in slowing gastric emptying (C_{450}) are related to MW by a relationship of the form $D = K(1/MW^p)$.

D refers to diffusion coefficients taken from *Handbook of Chemistry and Physics*, 51st edn. 1970/71.

C_{450} refers to the concentrations of acids giving recoveries of 450 ml. under standard conditions.

S is the slope of the line relating concentrations of acid in meals to the volumes of meals recovered under standard conditions. The values of p do not differ significantly

Method	$p \pm$ S.E. (standard error of regression constant)
$\frac{1}{C_{450}}$ vs. $\frac{1}{MWP}$	0·493 ± 0·052
S vs. $\frac{1}{MWP}$	0·649 ± 0·085
D vs. $\frac{1}{MWP}$ (MW 75–192)	0·55 ± 0·05
D vs. $\frac{1}{MWP}$ Acetic and citric acids	0·55

be constant for different acids as shown below:

C_{450} acetic acid × diffusion coefficient = $52 \times 1{\cdot}22 \times 10^{-5} = 63{\cdot}4 \times 10^{-5}$,

C_{450} citric acid × diffusion coefficient = $102 \times 0{\cdot}657 \times 10^{-5} = 67{\cdot}0 \times 10^{-5}$,

$$\frac{S\ \text{(acetic acid)}}{\text{diffusion coefficient}} = \frac{5{\cdot}00}{1{\cdot}22 \times 10^{-5}} = 4{\cdot}10 \times 10^{5},$$

$$\frac{S\ \text{(citric acid)}}{\text{diffusion coefficient}} = \frac{2{\cdot}66}{0{\cdot}657 \times 10^{-5}} = 4{\cdot}10 \times 10^{5},$$

All these numerical values, based on C_{450} and S which are to some extent independent, are remarkably consistent with the diffusion hypothesis.

Reservations on the diffusion hypothesis

Although it seems almost self evident that the influence of molecular weight upon the effectiveness of acids must operate either through diffusion or through some form of molecular sieving, we have reservations about the quantitative treatment given above which will now be discussed. Table 5 of Hunt & Knox (1969) gives regression equations for the relation between C_{450} and mol. wt. for fourteen subjects. The slopes of these within-subject regressions vary considerably with the implication that the apparent diffusion constants for acids are not the same for all subjects. For those fourteen subjects the duration of the test was chosen in an attempt to obtain a recovery of about 200 ml. with water. This gave an opportunity to study the effect of acids over a considerable range of concentrations. It also meant that the rates of gastric emptying for different subjects during the test with water varied from 50 to 17 ml./min. However, there was no relation between the rates of emptying and the regression of C_{450} on mol. wt. There was however a correlation between the volume of meal remaining with water for a subject and his value of p in the within-subject relation

$$\frac{10^3}{C_{450}}\ vs.\ \frac{10^3}{MWP}.$$

There was also a correlation between a subject's volume of meal remaining with water and his value of p in the relation

$$S\ vs.\ \frac{1}{MWP},$$

where S is the slope of Fig. 1, with dimensions increase in volume of meal recovered m-equiv increase in concentration of acid in the given meal. Since we as experimenters decided the volume of the test meal which would be recovered with water by adjusting the duration of the test between

subjects, we have reservations at present about the implications of the close concordance of the numerical values of the computed power functions discussed above (Table 5).

In animals. B. F. Leek (personal communication) has been able to record from afferent neurones which respond to different acids applied to the abomasal mucosa in sheep. On the basis of the difference in latency with a variety of acids he concludes that free diffusion over a distance greater than 200 μ could account for his findings. This diffusion might be occurring in the gastric pits in his preparation.

An explanation of the approximate equality of action of strong acids on the receptors slowing gastric emptying

To the first approximation the slowing of gastric emptying by nitric, sulphuric and cyclamic acids was equal to that by hydrochloric acid. On reflexion it is apparent that no matter what strong acid is given by mouth, chloride ions of gastric secretion and of the secretions entering the duodenum will be present in the duodenal lumen. Suppose two parts of a solution containing 60 mN cyclamic acid is diluted by one part of pancreatic secretion containing HCO_3^- 60 mN, Cl^- 100 mN and Na^+ 160 mN, the resultant concentration of ions will be H^+ 20 mN, Cl^- 33 mN, cyclamate 40 mN and Na^+ 53 mN. On the assumption that ion pairs move towards the hypothetical titration site, there is sufficient concentration of chloride ions to cover the diffusion of the hydrogen ions. Thus the limit to diffusion of hydrogen ion will be mainly set by chloride ion since it is more mobile than the cyclamate ion. The receptor will therefore respond as though hydrochloric acid has been given. The more the dilution of the test meal by pancreatic secretion, the more relevant does the above explanation become since the concentration of chloride ion will rise in relation to that of cyclamate ion.

The effect of the threshold for hydrochloric acid

Hunt & Knox (1969) (Table 2) paid some attention to the question as to whether or not there was a threshold for the effect of various acids. For hydrochloric acid the results were consistent with there being a threshold at about 5–10 mN, but this threshold was not seen with the other eight acids studied, with the possible exception of lactic acid. These observations now appear to have relevance to the working of the receptor responding to acid.

It has been postulated that the receptor responds to all strong acids as though they were hydrochloric acid. But acids classed as weak, for example citric acid, give pH values as low as 2·2 at concentrations of 100 mN. This corresponds to a concentration of hydrogen ions of 6 mN. Since there is chloride ion present, it would be expected that the receptor would respond

as if it were exposed to 6 mN-HCl in addition to 100 mN citric acid. Since hydrochloric acid is about three times as effective per milliequivalent per litre as is citric acid, it would be expected that the relationship between mol. wt. and effectiveness of weak acids would be distorted at high concentrations of citric acid, and other weak acids with relatively low pKs such as lactic and tartaric. This would distinguish these acids from acetic, propionic, etc., which do not give pH values less than 3 at concentrations up to 100 mN. The threshold for hydrochloric acid discussed above prevents this effect and the receptor apparently receives its acid mainly by diffusion of the undissociated weak acid, the rate of access being inversely proportional to the molecular weight of the acid.

The increased effectiveness in slowing gastric emptying of strong acids with high molecular weights

Contrary to the findings with weak acids, the strong acids with high molecular weights were more effective in slowing gastric emptying than were those with low molecular weights. The increased effectiveness of cyclamic acid might be the result of the nauseating properties of the cyclamate ion. To test this view a regression was plotted, using the results of Table 4, between the concentrations of hydrochloric, nitric and sulphuric acids (omitting cyclamic acid) giving recoveries of 450 ml. and the molecular weights of the acids. Between-subject variance was eliminated by plotting each concentration as a percentage of the mean of the three concentrations of the strong acids giving recoveries of 450 ml. for that subject. The concentration giving a recovery of 450 ml. became less as the molecular weight of the acid increased ($P < 0{\cdot}02$), just as it did when cyclamate was included. It therefore seems likely that the increase in effectiveness with molecular weight is general and not caused by any nauseating effect of cyclamate.

It is possible that the larger anions of the acids with higher molecular weights might be acting on the duodenal osmoreceptors. From the results of Hunt & Pathak (1960) and from some unpublished results of experiments with sodium cyclamate it can be stated that the increase in volume of test meal recovered/m-osmole.l. increase in concentration of sodium sulphate or cyclamate is about 1 ml. This effect is of the right order, in terms of osmoreceptor stimulation, to account for the greater effectiveness of the strong acids with high molecular weights.

A model for the receptor responding to acid

To work in the way which we have so far discovered our model of the duodenal receptor shown in Fig. 5, appears to need two parts.

1. A component which makes weak acids with high molecular weights

less effective than those with low molecular weights: a molecular sieve, already postulated for the intestinal mucosa (Fisher, 1955), or an aqueous diffusion path would serve equally well to explain our findings. Because of the quantitative agreement between the diffusion hypothesis and the results in Figs. 3 and 4, and our difficulty in specifying the properties of the sieve numerically, the concept of a diffusion path is preferred.

Assuming that the diffusion path is aqueous, on the grounds that hexanoic acid with a high fat/water partition coefficient is not especially effective in slowing emptying, it is possible to speculate on the site of the

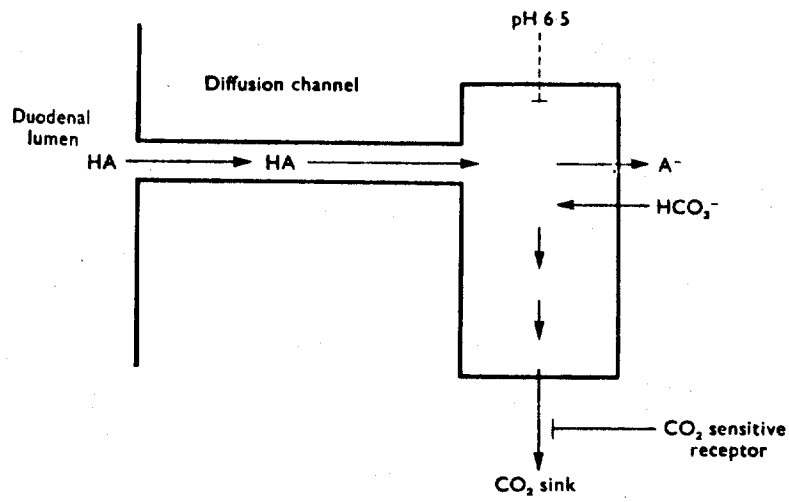

Fig. 5. Design for a hypothetical receptor responding to acids.

diffusion. To judge from the work of Harding & Leek (1972) with receptors in the abomasum of sheep, a diffusion pathway of about 200 μ would account for the difference in the latency of the afferent neuronal discharge with different acids. This is the order of magnitude of the pits in the gastric mucosa, or of the crypts of Lieberkuhn in the duodenal mucosa.

That there is a threshold concentration for the effect of hydrochloric acid might be explained by the walls of the crypts of Lieberkuhn being permeable to chloride ion so that some acid would be lost through them instead of diffusing to the titration site.

There are some facts which appear to be relevant to the walls of the diffusion pathway. Hexanoic acid has an oil/water partition coefficient of 38. Thus it should escape from the diffusion pathway if the walls were lipoprotein and should be less effective than expected on the basis of its molecular weight in the slowing of gastric emptying. In the event hexanoic

acid appears to have no special properties different from other weak acids. If the cells forming the walls of the diffusion pathway held their surface at say pH 6·5 the hexanoic acid in contact with the walls would be in the charged form, hexanoate, which is not especially soluble in lipoprotein and therefore would have no special escaping tendency. On the other hand, the proposal has been made above that hydrochloric acid does escape through the walls of the diffusion pathway. To conform with the proposal for limiting the escape of hexanoate it becomes necessary to postulate that hydrogen ions in close proximity to the walls of the diffusion pathway are neutralized by the inward diffusion of say sodium bicarbonate. This is perhaps a reasonable hypothesis since many of the cells lining the crypts of Lieberkuhn are goblet cells secreting mucus; but it would not explain a threshold concentration of about 5–10 mN for hydrochloric acid yet no such threshold for the majority of the other acids tested. Another possibility is the presence of a chloride-bicarbonate exchange across the wall of the diffusion pathway. The bicarbonate would then react with the hydrogen ion in the lumen of the diffusion path and the CO_2 produced could diffuse away through the walls of the crypts. It is possible that the hydrogen ion concentration activates the supposed exchange mechanism since the mean threshold computed by extrapolation for all strong acids is 3·5 (S.E. ± 1·96) m-equiv/l. and that for all weak acids is −3·1 (S.E. ± 2·02) ($P < 0·05$).

2. A component which titrates either weak or strong acids to pH 6·5 and indicates the concentrations. Our notion is that there is diffusion of an aliquot of the duodenal contents to a titration site in the depths of the mucosa, or possibly at the deep terminations of the crypts of Lieberkuhn which are up to 500 μ from the mucosal surface (R. H. M. McMinn, personal communication). The concentration gradient to the titration site is mainly that for the molecules of the intact acids, although the titration device is titrating protons, not anions. Since the system continues to distinguish between the acids by their molecular weights, there has to be a sink into which such anions as lactate, ascorbate, tartrate and citrate diffuse. These anions when taken by mouth are either excreted in the urine unchanged or to various degrees metabolized to bicarbonate. They can be absorbed and there need be no hesitation about postulating a vascular sink for such anions moving down the diffusion pathway.

The titration site is regarded as held at pH 6·5 by bicarbonate ions entering the chamber in exchange for the anions. The bicarbonate reacts with the hydrogen ions and produces a rise in partial pressure of CO_2 which diffuses out of the chamber into a CO_2 sink. A receptor responding to P_{CO_2} lying between the titration chamber and the sink for CO_2 gives a signal which varies with the rate of generation of CO_2 at the titration site. The receptor could be analogous to those responding to CO_2 in the system

controlling breathing. In essence this complexity turns all acids into carbonic acid and thus allows the response to be largely independent of the pK of the acid stimulating the receptor. Our view on the configuration of these components is shown diagrammatically in Fig. 5.

APPENDIX

Toxicity

Cyclamic acid (cyclohexylsulphamic acid)

At the time of these experiments cyclamic acid, as its calcium salt, was in wide use as a sweetening agent. The amounts received by our subjects were relatively small, since at the highest concentrations most of the meal was recovered. Cyclamate is now regarded as a potentially toxic substance when repeatedly ingested.

Nitric acid

The acute toxicity of nitric acid, like that of hydrochloric acid or sulphuric acid, depends mainly upon the concentration of hydrogen ion. Nitrate ion itself is relatively harmless. More than 500 m-equiv is a minimal acutely toxic dose. Thus nitric acid is as toxic as similar concentrations of endogenous hydrochloric acid. The maximal concentration of strong acids used in this work, 67 mN in HT, was less than half those used by many other workers. No untoward symptoms were produced apart from occasional nausea.

Nausea

In our experience materials which slow the gastric emptying of test meals usually produce nausea when given in high concentrations. But for corresponding action in slowing gastric emptying some substances are much more nauseating than others. For example, at relatively low concentrations glucose and potassium chloride, say 250 m-osmole/l. in test meals, slow gastric emptying equally, but at high concentrations potassium chloride is more likely to cause nausea than is glucose. When this happens the potassium chloride may appear to be slowing gastric emptying to a greater extent than glucose per m-osmole/l. In the present experiment we were studying the receptors responding to acid and slowing gastric emptying. We wished to exclude the action of any other type of receptor which might produce nausea, and slow gastric emptying. When nausea occurred we aspirated the gastric contents immediately. Cyclamic acid was the most nauseating acid and some of the subjects were excluded from the experiments because they were immediately nauseated by 10 mN cyclamic acid.

We are grateful to Professor A. G. Ogston F.R.S. for advice. We should like to thank our subjects for their cooperation, Miss Margaret Fisher for technical assistance, and Miles Laboratories, Elkhart, Indiana, for support.

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Johnson-'72
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Vol. 62, No. 1
Printed in U.S.A.

PEPSIN STIMULATED BY TOPICAL HYDROCHLORIC AND ACETIC ACIDS

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Normally the gastric mucosa is relatively impermeable to hydrogen ions. When, however, the barrier to diffusion is broken, acid enters the mucosa, causing damage. Back diffusion of H^+ and damage in dogs produces conditions similar to those existing in patients with gastric ulcer, and it has been suggested that the primary defect in gastric ulcer disease may be an abnormally permeable gastric mucosa. Pepsin secretion during the presence of noninjurious and damaging amounts of hydrochloric and acetic acid was studied in 4 dogs with vagally denervated pouches of the oxyntic gland area. Irrigation with 200 mN HCl caused a 40% rise in pepsin output, 250 mN HCl increased pepsin output 75%, and 300 mN HCl caused a 300% increase over control levels obtained during irrigation with 100 mN HCl. Pepsin outputs were directly proportional to the flux of H^+ out of the pouch. Irrigation with 100 mN acetic acid caused a 3-fold increase in pepsin output as well as increases in Na^+ and K^+ movement into the pouch fluid and H^+ movement out. Intravenous atropine (100 μg per kg-hr) prevented the acetic acid induced increase in pepsin secretion without significantly altering ion fluxes. It is concluded that: (1) back diffusion of H^+ stimulates pepsin output, (2) a local cholinergic reflex is probably involved in this stimulation, and (3) the increased pepsin output is due to actual secretion.

The gastric mucosal barrier and the alterations in the physiology of the barrier which occur during damage provide a meaningful model of the events which may lead to the formation of a gastric ulcer. The gastric mucosa is relatively impermeable to hydrogen ions. If, however, the barrier is weakened, back diffusion of acid occurs and damage results. Davenport[1-3] has shown that damaging the canine Heidenhain pouch (vagally denervated) with acetic or salicylic acid results in excessive movement of hydrogen ion out of the pouch and increased flux of sodium and potassium into the lumen. In addition, fluid accumulates in the pouch along with plasma protein.[4] If damage is severe, bleeding occurs. During damage, histamine is released into the mucosa[5] and the activity of the enzyme which forms histamine increases.[6] The higher levels of intramucosal histamine probably add to the damage by causing edema, fluid production, and increased mucosal permeability and acid secretion.

It has recently been shown that pepsin output increases when pouches are dam-

Received July 8, 1971. Accepted August 5, 1971.
These results were presented in part to the American Gastroenterological Association, Miami, Florida, May 1971.

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This study was supported by a grant from the G. A. Manahan Trust Fund and by National Institutes of Health Grant AM-14392.
The expert technical assistance of Louise H. Wyss is gratefully acknowledged.

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aged by high concentrations of hydrochloric acid in the presence of a subthreshold (for acid secretion) infusion of pentagastrin.[7] This study demonstrates that this increased pepsin output takes place in the absence of gastrin, is related to the back diffusion of hydrogen ion, and is secretory in nature.

Methods

Four dogs were surgically prepared with a gastric fistula drained by a Thomas[8] cannula and a vagally denervated (Heidenhain) pouch with a Gregory[9] cannula. These particular experiments were begun about 2 years after surgery. The animals were fasted for at least 18 hr but were allowed water before an experiment. At the start of an experiment the dogs were placed on tables where they stood quietly supported, in part, by slings. The gastric fistula was opened and the stomach was rinsed with saline. The fistula was left open for the duration of the experiment to prevent gastric juice from entering the duodenum. The metal cannula of the Heidenhain pouch was connected by rubber tubing to a reservoir with 50 ml of a control solution containing 100 mN HCl and 50 mN NaCl. The solution was allowed to enter and leave the pouch influenced only by gravity and pouch contractions. The level of fluid in the reservoir was about 20 cm above the pouch. At the end of each 15-min period the reservoir and the pouch were drained and the solution was replaced with 50 ml of fresh acid. Volumes of recovered fluid ranged from 48 to 51 ml, and, after the first two 15-min periods, which were not included in the experiment, were usually 50 ml.

Collections of the control solution continued for four 15-min periods. The pouches were then irrigated with 200 mN HCl, 250 mN HCl, 300 mN HCl, or 100 mN acetic acid plus 50 mN NaCl for four periods. In each case the 100 mN HCl control solution was made isosmotic with the test solution by the addition of NaCl. A final hour of collections with the control solution ended the experiment. At the beginning of an experiment in the acetic acid series an intravenous infusion of saline was started in a leg vein and maintained for the duration of the experiment at a rate of 60 ml per hr by a Harvard peristaltic pump. On some days atropine was added to the infusion to give a dose of 100 μg per kg-hr. Other experiments consisted entirely of irrigating the pouch with the control solution.

After each 15-min collection the volume of the sample was measured and the acid concentration was determined by titration to pH 7.0 with 0.2 N NaOH on an Autoburette and pH meter (Radiometer, Copenhagen). The Na^+ and K^+ concentrations of each sample were determined by flame photometry (IL model 143, Instrumentation Laboratory, Inc., Lexington, Mass.). A volume of each sample was immediately diluted so that it contained 50 mN HCl. The pepsin concentration of this sample was determined using a modification of the Anson[11] hemoglobin method and expressed as milligrams of pepsin per milliliter by reading the trichloroacetic acid supernatant fluid at 280 mμ and comparing it with solutions incubated with different pepsin standards (Hog pepsin, three times crystallized, Pentex Biochemicals, Kankakee, Ill.). Pepsin outputs were calculated, taking into consideration the dilution of the assay, and were expressed as milligrams of pepsin per 15 min. The data from the experiments with hydrochloric acid were expressed as the percentage of the mean of the two control periods during which the pouches were irrigated with 100 mN HCl and 50 mN NaCl. This was done to eliminate some of the variability between individual animals which had consistent but different basal levels of pepsin secretion. The mean pepsin value of the periods taken as 100% was 2.8 mg per 15 min.

Total amounts of Na^+, K^+, and H^+ in the recovered fluid were calculated for each period during the acetic acid experiments. The net flux of each ion was determined by subtracting the amount put in the pouch from the amount recovered and expressed as microequivalents per 15 min. Negative numbers indicate a net loss of an ion from the pouch, i.e., mucosal to serosal movement.

Experiments were done on alternate days and no more than three times a week. Within each group of experiments the order was randomly assigned. Results of the HCl study represent the means of three observations in each of four dogs. Following the completion of this series 1 animal developed a leaky pouch and was dropped from the study. Therefore, the acetic acid experiments were run on 3 animals and the data are expressed as the means and standard errors of the means of nine observations.

Results

Irrigation of the pouches with 200 and 250 mN HCl significantly increased pepsin output over that seen in response to 100 mN HCl ($P < 0.05$, periods 4 and 5, fig. 1). Irrigation with 300 mN HCl produced a

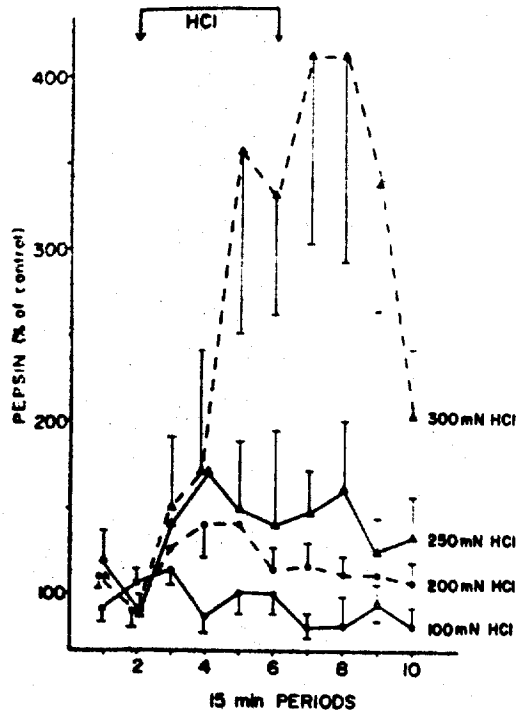

FIG. 1. Pepsin secretion as percentage of control from vagally denervated canine pouches. During periods 3 to 6 the pouches were irrigated with the concentrations of HCl indicated in the *margin*. During the remaining time, the irrigation solution contained 100 mN HCl plus NaCl to make the solution isotonic with the acid during periods 3 to 6. Means and standard errors of the means are shown for three observations in each of 4 dogs.

dramatic 3- to 4-fold increase in pepsin secretion. It can be seen in figure 1 that pepsin secretion was approximately the same during the control periods even though the osmolarity of the solutions differed. These relatively high concentrations of HCl were used to increase the diffusion gradient for H^+, thereby causing a flux of H^+ through the normal mucosa approximately equivalent to what would be expected if the barrier had been broken by an extraneous agent such as alcohol, acetic acid, or aspirin. These agents were avoided at the outset of the experiments in order to be able to attribute any stimulation in pepsin secretion to H^+ itself without having to worry about unphysiological anions or substances like alcohol which could stimulate on their own.

Pepsin secretion was directly proportional to the concentration of acid placed in the pouches for 100, 200, and 250 mN HCl (fig. 2). At 300 mN HCl this relationship no longer existed for pepsin output increased out of proportion to the increase in acidity. When, however, the data were replotted, pepsin secretion was found to be directly related to the net flux of hydrogen ion out of the pouch (*solid line*, fig. 2; $r = -0.997$). Thus, in addition to a large increase in pepsin secretion in going from 250 to 300 mN HCl, one also finds a concurrent increase in H^+ flux.

Irrigation of the pouches with 100 mN acetic acid (fig. 3) caused a 3-fold increase

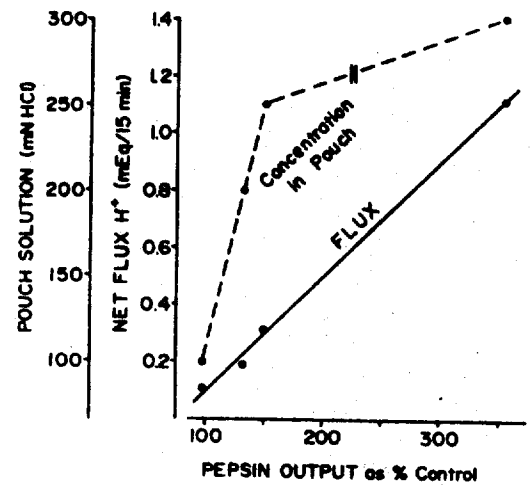

FIG. 2. Peak pepsin outputs from figure 1 plotted against the concentration of acid in the irrigating solutions and against the net flux of H^+ out of the pouch during the corresponding period.

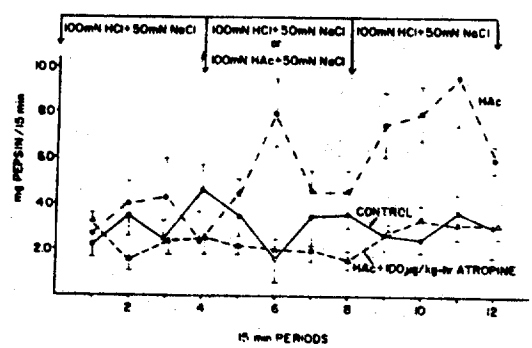

FIG. 3. Pepsin outputs in response to control period and irrigation with 100 mN acetic acid solutions. In one set of experiments atropine was infused intravenously. Means and standard errors of the means of three observations in each of 3 animals are shown.

in pepsin output over control periods before and during acetic acid irrigation (i.e., periods 4 and 6, $P < 0.01$). Following acetic acid irrigation, 100 mN HCl caused a further rise in pepsin secretion ($P < 0.01$, periods 9, 10, 11, 12) indicating that the barrier to ion diffusion had been disrupted by the acetic acid. Figure 3 also demonstrates that 100 μg per kg-hr atropine infused intravenously for the duration of the experiment completely blocked the increase in pepsin secretion in response to acetic acid.

The ability of acetic acid to damage the mucosal barrier was relatively unaffected by atropine. This is shown by the similarity in the volume changes and fluxes of Na^+, K^+, and H^+ during intravenous saline

TABLE 1. *Net changes across Heidenhain pouches of 3 dogs during intravenous saline infusion*[a]

Solution	Period	Volume	Na	K	H^+
		ml		*μEq*	
Control: 100 mN HCl and 50 mN NaCl	1	0	159	5	-654
	2	-0.5	-66	4	-381
	3	0.3	-79	3	-302
	4	0.5	25	2	-287
100 mN acetic acid and 50 mN NaCl	5	1.7	287	16	-653
	6	1.7	460	30	-916
	7	1.7	472	29	-950
	8	0.8	395	26	-914
100 mN HCl and 50 mN NaCl	9	1.3	404	21	-892
	10	1.0	334	18	-715
	11	1.3	326	15	-593
	12	2.2	364	14	-521

[a] Net volume and ion fluxes per 15-min period during the acetic acid experiments shown in figure 3. Saline was infused intravenously throughout. Numbers are means of three observations in each of 3 animals. Negative numbers indicate mucosal to serosal flux.

TABLE 2. *Net changes across Heidenhain pouches of 3 dogs during intravenous infusion of atropine (100 μg per kg-hr)*[a]

Solution	Period	Volume	Na	K	H^+
		ml		*μEq*	
Control: 100 mN HCl and 50 mN NaCl	1	0.3	209	10	-549
	2	0.1	50	7	-266
	3	-0.1	44	6	-307
	4	0.3	85	5	-325
100 mN acetic acid and 50 mN NaCl	5	0.7	248	20	-600
	6	1.3	425	26	-786
	7	0.7	367	24	-768
	8	0.8	334	21	-703
100 mN HCl and 50 mN NaCl	9	0.4	279	20	-894
	10	0.6	281	20	-608
	11	0.7	241	16	-624
	12	1.0	233	15	-519

Same as table 1 except that data were taken from the experiments in figure 3 in which atropine was infused throughout.

(table 1) and atropine (table 2) infusion. In other words, the pepsin output was the only entity altered by atropine.

Discussion

A previous study demonstrated that high concentrations of HCl could increase pepsin output in the presence of a background intravenous infusion of gastrin.[7] This work shows that pepsin output increases in response to the back diffusion of H^+ in the absence of gastrin stimulation. If the primary defect in some cases of gastric ulcer is, as Davenport[12] suggests, an abnormally permeable gastric mucosa, the concurrent increase in pepsin output could play a significant causative role in the production of gastric ulcers.

Large increases in pepsin output did not occur in response to HCl until sufficient acid was used to break the mucosal barrier to back diffusion. As is readily apparent in figure 2 the flux of H^+ did not increase out of proportion to the concentration put into the pouch until 300 mN HCl was used. Therefore, the threshold concentration of HCl which damages the barrier is somewhere between 250 and 300 mN. This is the same approximation arrived at by Davenport.[12]

The increased pepsin output in response to nondamaging concentrations of HCl is extremely interesting from the standpoint of the physiological control of pepsin secretion. These data indicate that the normal gastric mucosa contains a mechanism sensitive to H^+ which stimulates pepsin secretion, and we are currently involved in experiments to determine whether pepsin secretion is stimulated by the presence of physiological amounts of acid within the pouches.

The large increase in pepsin output in response to 100 mN acetic acid (fig. 3) indicates that the effect is not peculiar to damage by high (300 mN) concentrations of hydrogen ion. As Davenport[3, 4] has previously demonstrated, this concentration of acetic acid breaks the barrier to ion diffusion, resulting in the loss of H^+ from the pouch and a net gain of Na^+ and K^+ similar to what was observed in the current experiments (table 1). Although the barrier was broken by a much lower concentration of acetic acid than HCl, the relationship between pepsin output and H^+ back diffusion still holds. During acetic acid irrigation maximal pepsin secretion was roughly 300% greater than the control. This corresponded to a back diffusion of 916 μEq of H^+. In the flux curve drawn for HCl and pepsin output (fig. 2) a 300% increase in pepsin corresponds to the back diffusion of 900 μEq of H^+.

The observation that atropine blocks pepsin output during damage by acetic acid without affecting ion fluxes indicates that the increased output of pepsin is due to actual secretion. Since damage still occurs with atropine, the absence of the pepsin effect dissociates the source of pepsin from damage per se, that is, sloughed or broken cells. This observation also indicates the involvement of a local intramural cholinergic reflex in the stimulation of pepsin secretion.

From these experiments we conclude that (1) the back diffusion of H^+ increases the output of pepsin into the canine Heidenhain pouch, (2) the amount of pepsin secreted is directly related to the amount of H^+ lost from the pouch, (3) the increased pepsin output represents actual secretion, and (4) a cholinergic reflex is involved in the stimulation of this secretion. In addition, it is tempting to speculate that normal amounts of HCl in the stomach may actually enhance the physiological secretion of pepsinogen as well as activate it.

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Johnston-'65
Bib no. 203

Lancet 11(7421):1032-1035. 1965.

INHIBITION OF GASTRIN SECRETION IN THE HUMAN STOMACH

Effect of Acid in the Duodenum

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THE existence in the duodenum of a mechanism that can inhibit gastric secretion under experimental conditions is well established, both for the dog (Sokolov 1904, Day and Webster 1935, Code and Watkinson 1955, Sircus 1958, Andersson 1960, Wormsley and Grossman 1964, Konturek and Grossman 1965) and for man (Griffiths 1936, Shay et al. 1942, Johnston and Duthie 1964, 1965). We have shown previously that histamine-stimulated gastric secretion in normal men and patients with duodenal ulcer was inhibited by perfusing the proximal duodenum with decinormal hydrochloric acid, and suggested that this inhibition was partly humoral in nature. Considerable doubt persists about the relevance to human physiology of the findings of these investigators since both the stimuli, such as histamine, and the inhibitors in the duodenum, such as 0·1 *N* hydrochloric acid, have been

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claimed to be unphysiological. This paper describes the inhibition by duodenal acidification of gastric secretion stimulated in human volunteers by the pure gastric hormone, gastrin II.

Method

The subjects were eleven healthy young men and three patients with chronic duodenal ulcer. After an overnight fast, two tubes were passed into the stomach, by the nasal route. With the aid of the image intensifier, one was placed in the most dependent part of the stomach, and the other in the second part of the duodenum. Since the latter tube was biluminial, it was possible to perfuse the second part of the duodenum with fluid, which entered at the tip of the tube and was sucked out again via the proximal suction holes. Inevitably, some of the infused fluid passed on down the duodenum. This system reduced and usually entirely prevented reflux of duodenal contents into the antrum of the stomach, while the continuous suction on the tube prevented distension of the duodenum and yielded copious volumes of duodenal juice for measurement of pH and osmolarity. A detailed account of the method has been published (Johnston and Duthie 1964).

The tubes having been placed in position, gastric secretion was stimulated by infusing gastrin II intravenously for 3 hours, at a constant rate from a Palmer pump. It was found that a dose of 2·5 μg. per hour elicited a level of secretion that was about 45% of the maximum. When the gastric secretory response had reached a plateau, after 60 to 105 minutes, perfusion of the duodenum was commenced, using either 0·1 *N* hydrochloric acid labelled with the inert marker polyethylene glycol (P.E.G.), 0·5%, or, as a control, 0·9% saline solution with P.E.G. The mean volume used was 140 ml. per 15 minutes (range 50–200 ml.). The use of P.E.G. permitted accurate calculation of the amount of reflux into the stomach, from a consideration of the respective concentrations of the marker found in the gastric and duodenal aspirates. The duodenal perfusion usually lasted 45 minutes. After this the test was continued for a further 45 minutes or so, making the total length of the test about 3 hours.

Five more tests were performed in which 2·5 μg. of gastrin II per hour were infused intravenously for 3 hours without duodenal perfusion.

Continuous suction was applied to both gastric and duodenal tubes; separate specimens were collected for each 15-minute period. A radiograph at the end of most tests showed that the tubes had not moved.

The following estimations were done:

Gastric juice.—Volume, hydrogen-ion concentration, and titratable acidity (Moore and Scarlata 1965) using the indicators Töpfer's reagent and phenolphthalein, pepsin by the method of Hunt (1948), and concentration of P.E.G. (Hyden 1955).

Duodenal aspirate.—Volume, pH, hydrogen-ion concentration, and titratable acidity, P.E.G. concentration, and osmolarity by the method of freezing-point depression using an 'Advanced' osmometer.

Results

DUODENAL ACIDIFICATION

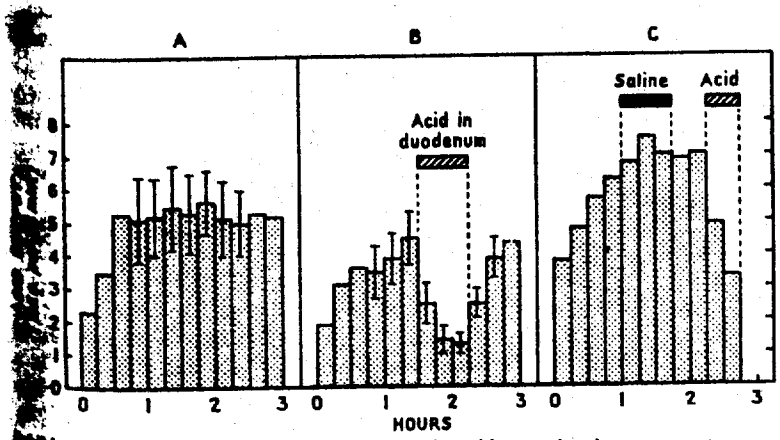
Secretion of Acid

The gastric secretion of acid in response to gastrin II was inhibited by duodenal acidification in ten of eleven tests. The inhibition affected the volume, concentration, and output of acid. Eight of the ten tests were on healthy subjects, and two tests were on patients with duodenal ulcer.

The onset of inhibition was rapid. It was apparent within 15 minutes of the start of duodenal perfusion in nine of the ten tests in which inhibition occurred. It occurred within 5 minutes in each of two tests in which the gastric aspirate was collected in 5-minute portions. In two other tests (nos. 1 and 8, see accompanying table) the inhibition was so obvious within 5 minutes that it could be appreciated even though the 15-minute samples were not divided into 5-minute specimens. Inhibition was greater in the second 15 minutes of duodenal perfusion than in the first 15 minutes, but then remained at about the same level in the third 15-minute period. When perfusion of the duodenum with acid was stopped, the volume of gastric acid secretion began to increase at once. The increase in concentration of gastric acid took place more slowly, as shown in the accompanying figure. In about half the tests, control levels of secretion were regained after 30 to 45 minutes.

In the ten tests showing inhibition, the mean decrease in volume was 52%, in concentration 29%, and in output of acid 67%: all these changes are highly significant ($P < 0·001$). In making these calculations, the gastric secretion for the 2nd and 3rd 15-minute periods of the duodenal perfusion was compared with that for a control period which was usually the 30-minute period immediately preceding duodenal perfusion with hydrochloric acid.

In one test (on a normal subject) there was no inhibition. Two possible explanations for failure were that the stimulus—in this case 5 μg. of gastrin II per hour—elicited very high levels of secretion (about 10 mEq. per 15 minutes); and that, although the duodenal tube was verified by a check X-ray to be well sited in the second part of the duodenum, the duodenal aspirate remained persistently alkaline because the infused hydrochloric acid was neutralised by bile and pancreatic juice. Weak inhibitory influences could not prevail against the strong stimulation.



Inhibitory effect of acid in duodenum on gastric acid secretion in response to pure gastrin II, 2·5 μg. per hour.

A. Mean of 5 control experiments, with no acid in the duodenum.

B. Mean of 10 tests, showing inhibition by acid in duodenum.

C. Mean of 4 tests: no effect from physiological saline solution in the duodenum, but later inhibition by acid. Vertical lines represent ± one standard error of the mean.

Pepsin

The changes in output of pepsin accorded roughly with the changes in acid secretion. A highly significant decrease in output of pepsin occurred ($P < 0·001$). There was no significant change in concentration of pepsin.

Details of the tests are given in the accompanying table and figure. Two other tests have been excluded because of excessive reflux of duodenal contents into the stomach during the perfusion with acid. In both, inhibition was evident despite the reflux. In another test, on a normal

INHIBITION BY ACID IN THE DUODENUM OF THE GASTRIC RESPONSE TO GASTRIN II GIVEN INTRAVENOUSLY

Test no.	Diagnosis	Acid: Output (titratable acidity, mEq. per 15 min.)			Acid: Concentration (mEq. per litre)			Pepsin: Output (units per 15 min.)			Pepsin: Concentration (units per ml.)			Reflux, duodenum to stomach (ml.) in the three 15-min. periods of perfusion			Osmolarity in duodenum (mosm. per litre)		pH (lowest reached in duodenum)
		Control	Acid in duodenum	% inhibition	Control	Acid in duodenum	% inhibition	Control	Acid in duodenum	% inhibition	Control	Acid in duodenum	% inhibition	1	2	3	Control	During perfusion	
1	N	0·62	0·08	87·1	35	30	14·3	..	..	..	..	..	..	0	0	0	306	167	2·0
2	N	4·96	1·34	73·0	89	48	46·1	3541	1758	50·4	63·2	62·8	0·6	8	1	2	293	160	2·4
3	N	4·20	1·68	60·0	105	83	20·9	3276	1961	40·1	81·9	97·7	−19·3	0	0	0	285	167	2·0
4	N	2·56	0·57	77·7	64	42	34·4	1510	891	41·0	37·8	50·5	−33·6	0	0	0	258	218	7·6
5	N	5·38	2·33	56·7	108	81	25·0	3480	2120	39·1	70·2	76·6	−9·1	3	1	0	318	151	1·9
6	N	1·86	1·04	44·1	64	40	37·5	2753	2437	11·5	92·0	80·4	12·6	0	0	0	297	178	9·0
7	N	4·91	1·56	68·2	111	77	30·6	3755	1546	58·8	78·1	82·9	−6·1	2	0	0	287	155	2·0
8	N	5·30	0·31	94·1	98	74	24·5	3370	378	88·8	63·0	63·0	Nil	0	0	0	262	179	6·7
9	D.U.	7·35	2·86	61·1	143	110	23·1	6187	3562	42·4	120·1	113·0	5·9	5	0	..	..	..	6·0
10	D.U.	4·03	2·30	42·9	85	55	35·3	4113	2533	38·4	85·4	56·8	33·5	1	3	..	..	..	2·2
Mean	..	4·12	1·41	66·5	90·2	64·0	29·2	3199	1719	45·6	76·9	76·0	−1·7	..	..	..	288	172	..
Significance of difference ..		P<0·001			P<0·001			P<0·001			Not significant			..	..	..	P<0·001		..

Dose of gastrin II was 2·5 μg. per hour, except in no. 1 (5 μg. per hour) and no. 4 (9 μg. per hour).
N = normal subjects. D.U. = patient with duodenal ulceration.

subject, acid put into the duodenum an hour after the intramuscular injection of 15 μg. of gastrin II led to a prompt decrease of 37% in output and 32% in concentration of acid: in a control experiment on another subject who was given the same dose of gastrin, but no acid into the duodenum, no spontaneous decrease in secretion took place at this time.

CONTROL STUDIES

Saline Perfusion

Physiological saline solution introduced into the duodenum in four tests did not inhibit the gastric response to gastrin, whereas acid introduced later in each test led to significant inhibition (see figure).

3-hour Gastrin Infusion

In the control experiments in which gastrin II, 2·5 μg. per hour, was infused intravenously for $2^1/_4$–3 hours, the mean output and concentration of acid in the 7th, 8th, and 9th 15-minute periods were 102% and 97% of the output and concentration in the 4th, 5th, and 6th 15-minute periods (see figure). Since no spontaneous decrease in secretion took place at this time, the null hypothesis was used in calculating the degree of significance of the inhibition in the ten tests.

PERFUSION

The presence of a stout wire stylet inside the duodenal tube imparted such rigidity to it that the intubation procedure took only 3 or 4 minutes in most cases. Suction on the tube produced large volumes of duodenal juice and this was the crucial factor in preventing reflux of duodenal contents into the stomach. The volume of acid infused into the duodenum (mean: 140 ml. of 0·1 *N* hydrochloric acid per 15 minutes) is no doubt greater than that present in the chyme entering from the stomach after a meal, but it must be remembered that much of the acid was sucked back out again almost immediately, the mean rate of recovery of duodenal juice being 93 ml. per 15 minutes during the perfusion period.

There was no reflux of duodenal content into the antrum in five of the ten tests showing inhibition, and very little in three others (table I). In cases 2 and 9, reflux of 8 ml. and 5 ml. in the first 15 minutes of duodenal perfusion did not invalidate the results, because the fluid was alkaline and could not have invoked the antral inhibitory mechanism and the results of the first 15 minutes were not used to calculate the degree of inhibition.

During perfusion of the duodenum with acid, the pH of the duodenal aspirate was by no means always acid, and inhibition sometimes occurred when the aspirate had a pH greater than 6. Details of the pH readings are given in the table.

There was a highly significant decrease in osmolarity of duodenal contents (P < 0·001) during the perfusion with acid, from a mean of 288 to 172 milliosmoles per litre (see table).

Discussion

Gastric secretion in response to the natural hormone gastrin II has been inhibited by acid perfusion of the duodenum in normal volunteers and in men with duodenal ulceration. Thus the hypersecretion of acid in these patients with duodenal ulcer is unlikely to be a result of defective duodenal inhibition of gastric secretion. The same broad conclusion was reached by Hunt (1957) after tests of gastric emptying and secretion in patients with duodenal ulcer, though he did find that in a minority of such patients the osmoreceptor mechanism in the duodenum was defective. This is in contrast to the suggestion of Shay and his colleagues (1942) that defective duodenal inhibition was an important cause of the gastric hypersecretion.

pH in the Duodenum

Pincus et al. (1942) suggested, on the basis of animal experiments, that a pH of 2·5 or less must be reached in the duodenum before gastric secretion would be inhibited. In the present study, in six of the ten tests showing inhibition the duodenal pH was 2·6 or less; in the remaining four tests the pH of the duodenal aspirate was greater than 5·8, although free acid was detected occasionally over short periods even in these four.

A zone of high acidity was probably created around and distal to the tip of the duodenal tube, whereas more proximally, at the level of the suction holes, the infused acid was mixed with the bile and pancreatic juice which had been discharged in copious amount into the duodenum in response to the presence of the acid. We have no direct evidence that the critical level of pH in the duodenum is the same in man as in the dog.

In using decinormal hydrochloric acid to perfuse the duodenum we have attempted to imitate normal conditions. The proximal part of the human duodenum is

known to be highly acid after meals (Atkinson and Henley 1955, Rovelstad and Maher 1962, Rhodes et al. 1965). It is of interest in this connection that when subject no. 9's own gastric acid (concentration 120 mEq. per litre) was gravitated into the duodenum, the response of his stomach to intravenous gastrin was inhibited.

Osmolarity in the Duodenum

The duodenal perfusion with hydrochloric acid brought about significant changes not only in the hydrogen-ion concentration but in the osmolarity of the duodenal contents (from 288 to 172 milliosmoles per litre). Two pieces of evidence suggest that the change in pH was the important factor in causing inhibition of gastric secretion. Firstly, Hunt (1956), in studying the alimentary osmoreceptor mechanism in man, found that solutions of osmolarity 100–160 milliosmoles per litre left the stomach at the maximal rate, while as osmolarity increased from 160 to 350 milliosmoles per litre the rate of gastric emptying slowed progressively. It would appear that a solution of osmolarity 172 milliosmoles per litre would be unlikely to elicit major changes in gastric motility, or secretion. Secondly, Sircus (1958), working with dogs, reported that inhibition of gastric secretion was produced by solutions of osmolarity exceeding 275 or less than 50 milliosmoles per litre, so that again a solution of 172 milliosmoles per litre would not be expected to inhibit gastric secretion.

In experiments such as ours, the possibility that "inhibition" of gastric secretion is merely due to increased losses of gastric acid via the pylorus must be considered: this, however, is a most unlikely explanation for our findings, because duodenal acidification is known to inhibit gastric motility, raise the tone of the pyloric sphincter, and increase motility in the duodenum (Schapiro and Woodward 1955, Hunt 1957, Brink et al. 1965).

Subjective Factors

Of the 10 subjects with inhibition, 4 experienced nausea soon after the perfusion of the duodenum with 0·1 *N* hydrochloric acid began. It was transient, and always eased by running the acid into the duodenum too quickly. Inhibition followed the same pattern in these subjects as in the other 6 without nausea. Furthermore, inhibition persisted after the nausea passed off. It would appear that the only significant inhibitory factor was acid in the duodenum. On two other occasions nausea was produced when large doses of gastrin II (20 and 50 μg.) were given intravenously over 10 seconds: the gastrin produced further stimulation of gastric secretion (which already was on a plateau in response to 2·5 μg. of gastrin II per hour) instead of the inhibition which might have been expected from the work of Gillespie and Grossman (1963) on dogs. Larger doses of gastrin might have produced inhibition. Apart from these two instances, gastrin has produced no side-effects throughout the series of experiments.

Histamine and Gastrin

Inhibition by acid in the duodenum of the gastric secretion elicited by histamine differed in one major respect from inhibition when gastrin was the stimulus. With stimulation by histamine, the inhibition affected volume and acid output only, the concentration of acid secreted by the stomach remaining unaffected: with gastrin as the stimulus, the concentration of acid was also diminished. Though with gastrin the inhibition was greater (67%, as against 57% with histamine) and apparently more readily elicited (in 14 subjects, as against 12 of 16), these differences may well not be significant, because of the greater efficiency of the method in the present series and the lower levels of gastric secretion elicited in the control period. Differences in the timing of the inhibition may likewise not be significant: these were a less rapid onset of the maximal degree of inhibition when gastrin was the stimulus, and a slower increase to control levels when duodenal perfusion had ceased. These less abrupt changes in secretion may mean that humoral factors were of greater significance in eliciting the inhibition when gastrin was the stimulus. Such a suggestion finds some support in the results of canine experiments (Greenlee et al. 1957, Kennedy and Hallenbeck 1963, Gryboski and Menguy 1964) which show that secretin (which is known to be released when acid enters the duodenum) inhibits food-stimulated, but not histamine-stimulated, gastric secretion. Against this must be set the finding by Wormsley and Grossman (1964) that secretin did inhibit histamine-stimulated pouch secretion in dogs; but even these workers did not consider that secretin was the only inhibitor released from the duodenum by endogenous acid.

Our finding that acid in the duodenum inhibits gastric secretion in response to intravenous administration of gastrin II, agrees well with previous results with exogenous gastrin in animals (Wormsley and Grossman 1964). These authors also showed that secretin may have a role in this inhibition. Other evidence was obtained in man by Kamionkowski et al. (1964) who inhibited broth-stimulated gastric secretion by intravenous injection of secretin. On this basis, release of secretin by acid put into the duodenum in the present study may have been important in bringing about the inhibition, but the relative roles of the duodenal hormonal substances and of the vagus nerves have not been defined.

With the demonstration that the introduction of decinormal hydrochloric acid into the second part of the duodenum leads to inhibition of gastric secretion in response to pure gastrin II in normal men, the relevance of duodenal inhibition to human physiology and surgery comes nearer to being established. Inhibition of food-stimulated gastric secretion can be forecast. But one question is still unanswered: in man, what changes take place in the acidity and tonicity of the duodenal contents after normal meals, and what effect do such changes have on the secretory response of the stomach to the meals?

Summary and Conclusion

In normal men and patients with duodenal ulcer, gastric secretion of acid and pepsin in response to pure gastrin II is significantly inhibited by the introduction of decinormal hydrochloric acid into the second part of the duodenum. It is suggested that duodenal inhibition of gastric secretion is elicited by the onward passage of the acid gastric contents after a meal, is thus of physiological importance, and should be preserved if possible in the performance of operations for duodenal ulcer.

We thank Prof. R. A. Gregory for his generous gift of pure gastrin II; and Mr. F. D. Naylor, Mr. David Robinson, Miss Zena Hinchliffe, Miss Hazel Peck, and Miss Jennifer Senior, for technical assistance, Dr. A. Lant for the osmolarity estimations, and the staff of the radiology department, Sheffield Royal Infirmary, for their help. We are greatly indebted to the volunteers.

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Karon-'51
Bib No. 207

Gastroenterology 17:445-449. 1951.

PYLORIC STENOSIS CAUSED BY THE INGESTION OF A CORROSIVE ACID, SIMULATING GASTRIC CARCINOMA: REPORT OF A CASE

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The late sequelae of the ingestion of corrosive acids may mimic a malignancy very closely and confuse the diagnosis if one is not cognizant of the fact that the ingestion of a corrosive acid may produce a high grade pyloric obstruction with achlorhydria in four to six weeks without damage to the esophagus. This clinical syndrome was first described in 1828 by Robert[1]. There is an excellent recent review of the literature by Gray and Holmes[2]. Boikan et al.[3] and McLanahan[4] also discuss the subject thoroughly. Only 140 cases have been reported in the world literature and of these only 12 appear in the American literature[5]. The monographs on gastro-enterology and toxicology[6] have almost uniformly omitted a good description of the characteristic sequence of events after recovery from the acute effects of swallowing corrosive acids. Esophageal stricture is a well recognized complication following the ingestion of lye; only about 20% of these cases with esophageal stricture have associated pyloric stenosis[6]. The reverse situation following the ingestion of corrosive acids is not as well known. The usual complication is progressive pyloric stenosis; an associated esophageal stricture develops in only about 20% of the cases[2,7].

CLINICAL FEATURES

There are usually two reasons for the ingestion of a corrosive acid. The acid may be taken in an attempted suicide; more often it is swallowed by error, frequently being mistaken for whiskey.

The immediate clinical manifestations are burning of the mouth and lips, vomiting, and sometimes hematemesis. If the patient does not die from the immediate effects, vomiting ceases in a few days, oral lesions disappear and within a week or ten days the patient feels well and apparently has recovered.

Symptoms due to pyloric obstruction: vomiting, epigastric pain which is usually a mild sense of fullness and pressure after meals, relieved by vomiting, develop in approximately three or four weeks. The free HCl is generally low or absent. There is loss of weight. The roentgenogram reveals almost complete pyloric obstruction. Because of the infrequent association of esophageal injury the picture is frequently mistaken for carcinoma of the stomach, even though a history of ingestion of acid previously is known to the radiologist. The correct diagnosis may be more difficult if this information is not made available.

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If the acid is swallowed on a full stomach, food prevents contact with all except the lesser curvature and pars pylorus. Thus, there results a pyloric stenosis, scarring of the lesser curvature and some antral involvement. If on the other hand, the acid is taken when the stomach is empty, the ingested fluid comes in more general contact with the gastric mucosa and especially with the pars pylorica and pars media. This may lead to a perforation of the stomach or a diffuse gastritis and later pyloric and antral constriction, with subsequent linitis plastica or hour-glass type stomach[8].

TREATMENT

The end result of ingestion of acid is permanent pyloric obstruction that will lead to death unless corrected surgically. The earliest successful treatment was pyloroplasty. This was replaced by gastro-enterostomy which has been used by the majority of surgeons. Recently Gray and Holmes[2] advocated partial gastrectomy as the treatment of choice. The latter operation was used successfully in our patient.

REPORT OF CASE

A 57 year old white man with depressive tendencies was first admitted to the Menorah Hospital as a private patient on April 30, 1949, one day following the ingestion on an empty stomach of 45 cc. of muriatic acid diluted with 150 cc. of water taken with suicidal intent. He immediately developed burning pain in his mouth, throat and epigastrium. The morning of admission he vomited a small amount of blood and complained of epigastric pain and nausea and had a soft tarry stool. There were only minimal superficial burns on his lips and buccal mucosa. The pharynx apparently was not involved. The blood pressure was 134/80, pulse 70 and respirations 18. The maximum temperature was 99.8 F. The patient rapidly improved on an ulcer regime of milk and cream mixture, sedation and anti-spasmodics; on the fifth day he was entirely asymptomatic; he left the hospital April 6, 1949.

He was next seen as a clinic patient on June 9, 1949; the long history of gastrointestinal complaints, achlorhydria (gastric analysis had not been performed prior to ingestion of HCl) and roentgen evidence of severe pyloric obstruction suggested a diagnosis of peptic ulcer with obstruction or gastric carcinoma. He was now admitted to the medical service of the same hospital June 17, 1949 about seven weeks after the first admission. He had been vomiting after meals for the past 2 or 3 weeks. The vomitus contained undigested food that had been eaten the day before. Other symptoms included poor appetite, vague abdominal distress, definitely not of ulcer character, and recently (preceding 3 weeks) abdominal distress described as fulness immediately after eating and not relieved by food. He had lost about 7 lbs. in weight since the previous admission.

Physical examination was normal. Laboratory studies: The stomach was aspirated twice daily with the removal of 500 cc. to 1100 cc. Roentgenologic examination of the upper gastrointestinal tract revealed a normal esophagus, constant narrowing at

the distal portion of the antrum, irregularity of the mucosa and almost complete pyloric obstruction (fig. 1). Impression: "chronic indurated ulcer or neoplasm, a neoplasm favored." Gastroscopic examination disclosed a "narrow shallow ulcer covered with white fibrin on the lesser curvature from the angulus to the antrum not extending into the pylorus. The pyloric opening was visualized but was not seen to open during the examination. Remainder of the stomach showed old petechiae and patches of erythema. Conclusion: 'benign ulcer' ". Gastric analysis on three occasions, two following histamine (.5 mg. histamine base) yielded no free HCl and total acid up to 20°. The feces were negative for occult blood. Blood count, RBC 4,750,000 per cu. mm., Hb. 14.7 gm. per 100 cc. of blood, WBC 8,600 per cu. mm.

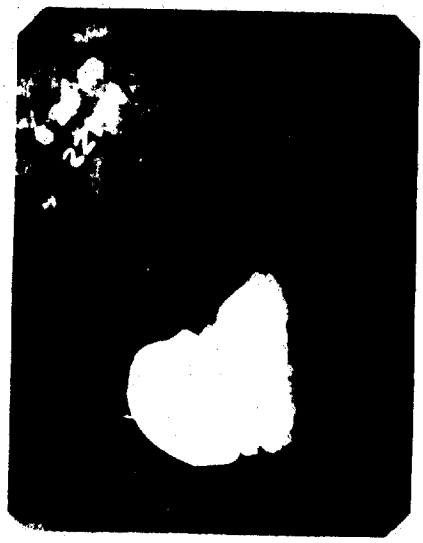

FIG. 1

FIG. 2

with a normal differential. On June 28, 1949 a subtotal gastric resection was performed, ⅔ of the stomach being removed.

Pathologic report

Macroscopic: The antrum, 2.5 cm. proximal to the pylorus, contains an ulcerated lesion with well demarcated circinate edges. The mucosa of the lesion is not fixed. The floor of the ulcer is indurated, hemorrhagic and granular. The distal 2.5 cm. of the prepyloric antrum show marked stenosis, hypertrophy of wall and prominent longitudinal rugae of mucosa (fig. 2).

Microscopic: Antrum: The ulcer generally extends into the submucosa but not deeper. The base of the ulcer is heavily infiltrated with inflammatory cells (chiefly lymphocytes and plasma cells). The veins at the base of the ulcer are numerous, dilated and congested. To the sides of the ulcer the following are noted: atrophy to

polypoid hyperplasia of the mucous membrane, hypertrophy of muscularis mucosae, fibrosis and widening of the submucosa with patchy inflammatory cell infiltration, hypertrophy of muscularis propria with associated fibrosis and inflammatory cell infiltration. The inflammatory cell infiltration is similar at all locations. At the pylorus the gastric and duodenal mucous membranes are essentially free of significant change.

Fundus: Focally there are dilated glands. The parietal cells are reduced in number but not absent. There are focal infiltrations of acute and chronic inflammatory cells in the muscularis propria and the serosa. In one section is an acute ulcer extending into the submucosa.

Two lymph nodes show mild reactive chronic lymphadenitis.

Diagnosis: Chronic severe gastritis of antrum with large, relatively superficial ulceration, and hypertrophy and stenosis of prepyloric antrum.

The patient made an uneventful recovery and was discharged from the hospital one month after admission. Nine months later he reported that he had had no gastrointestinal symptoms of any kind and had gained 20 lbs.

COMMENT

This 57 year old man entered the hospital with a history of epigastric distress not characteristic of ulcer for at least 10 years, becoming more pronounced in the past year, long before the ingestion of acid. He gave no history of the suicidal attempt to the interne on the second admission and we became cognizant of it only after a review of his previous record. His previous difficulties had been considered to be functional in origin by his past physicians but no roentgenologic studies were made prior to the acid ingestion. He had been given electro-shock therapy for a depressive state several years before. In view of the evidence of nearly complete pyloric obstruction, histamine achlorhydria, the gastroscopic visualization of an antral ulcer, and the apparently minimal trauma to his mouth and pharynx with no involvement of the esophagus after the ingestion of acid together with his rapid recovery, the significance of the ingestion of HCl was not at first appreciated and a tentative diagnosis of a gastric malignancy was made. Hence, it was decided to do a gastric resection.

SUMMARY

1. This paper is based on the report of a case in which pyloric stenosis without esophageal stricture developed approximately one month after the ingestion of 45 cc. of hydrochloric acid.

2. The ingestion of caustic alkalis produces damage primarily to the esophagus; pyloric stenosis develops only occasionally (20%), whereas, the ingestion of corrosive acids produces its primary effect on the stomach. This may result in perforation of the stomach and death. If the patient recovers from the acute gastritis, he often develops a severe pyloric obstruction and achlor-

hydria in from four to six weeks. Approximately 20% of the patients with pyloric stenosis also develop esophageal stricture.

3. The pyloric stenosis may be so severe as to result in death unless corrected surgically.

4. The clinical picture produced by pyloric stenosis following acid ingestion may closely simulate gastric malignancy.

ACKNOWLEDGMENTS

Dr. Leo Pollock, Department of Surgery, performed the gastric resection.

Dr. Jack Wolf, Department of Medicine, performed gastroscopy.

Dr. Hilliard Cohn, Department of Pathology, was responsible for the pathologic studies.

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Kinard-'35
Bib no. 216

Amer. J. Med. Sci. 190(2):242-244. 1935.

FAILURE OF INTRAVENOUS HYDROCHLORIC ACID TO SHORTEN ANESTHESIA.*

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In view of the widespread interest in the announcement that intravenous injections of N/10 hydrochloric acid promptly terminate anesthesia and because of the great practical value of such a discovery, it was deemed advisable to carry out controlled experiments testing the accuracy of this report.

It seems that Miss Moorman,[1] of Joplin, Mo., having found that intravenous injections of hydrochloric acid terminated anesthesia in animals, told Dr. MacGilvra who, with a Kansas City laboratory, verified the results. The injections were then used successfully in a patient in an emergency. In 7 other instances, anesthesia in patients was terminated by the use of the acid.

MacGilvra[2] reported that the hydrochloric acid injections had accelerated the recovery of rabbits, guinea pigs, rats, monkeys, and 8 human beings under ether anesthesia and 1 human being under avertin anesthesia. No details were given except in the case of the patient under avertin anesthesia. Ellison and MacGilvra[3] later discussed the remarkable recovery of this patient and suggested further probable uses of the hydrochloric acid injections.

Shambaugh and Boggs,[1] however, were unable to accelerate recovery from anesthesia in dogs anesthetized with ether or avertin or nembutal by the intravenous injection of N/10 hydrochloric acid. Neither were they[5] able to shorten the recovery period of guinea pigs, rabbits, dogs, monkeys and baboons from ether, pentobarbital sodium and tribrom-ethanol narcosis. Control animals were used in these experiments.

In view of these differences it was decided to carry out carefully controlled experiments on dogs and rabbits anesthetized with ether, pentobarbital sodium, and paraldehyde[3] to study the effect upon the recovery of the animals following the intravenous injection of N/10 hydrochloric acid.

Methods. Mature, well-nourished dogs and rabbits were used in all experiments. In some cases the animals were subjected to anesthesia several times, but only after a rest of at least 7 days.

* Read before the South Carolina Academy of Science, Columbia, S. C. April 6, 1935.

Pentobarbital sodium (nembutal) was given by inhalation, and the paraldehyde was given by stomach tube. Ether was administered evenly to a point where respiratory failure appeared imminent. The N/10 hydro-

TABLE 1.—ETHER.*

Animal.	Weight, kg.	HCl, N/10 cc.	Attempts to stand, minutes.	Stands, minutes.	Walks, minutes.
Dog	17.6	10.0	10.0	11.0	11.5
"	17.7	10.0	10.0	10.5	11.0
"	6.4	1.5	9.0	10.0	10.5
"	7.3	5.5	7.0	10.5	11.5
"	10.0	10.0	9.0	11.5	12.5
"	9.5	8.0	7.5	12.5	19.0
"†	6.0	0.0	5.0	7.0	7.5
"†	7.4	0.0	4.0	5.5	6.0
"†	16.2	0.0	5.0	7.0	8.0
"†	18.2	0.0	5.0	6.0	6.5
"†	10.1	0.0	19.0	21.0	21.0
Rabbit	2.2	1.0	3.0	9.0	9.5
"	2.3	2.0	4.0	8.5	11.0
"	2.8	2.0	9.0	11.5	20.0
"	1.9	2.0	9.0	14.0	15.0
"	2.7	2.0	5.5	18.0	21.0
"	2.7	2.0	5.0	7.5	8.0
"†	2.7	0.0	4.0	4.2	5.5
"†	1.6	0.0	10.0	11.0	11.5
"†	2.4	0.0	2.5	5.2	5.5
"†	1.9	0.0	4.5	6.5	18.0
"†	2.2	0.0	5.5	8.5	9.0
"†	2.4	0.0	6.5	7.0	8.0

* Administered by inhalation.
† Control.

TABLE 2.—NEMBUTAL.*

Animal.	Dosage, gr.	Weight, kg.	HCl, N/10 cc.	Attempts to stand, hours.	Stands, hours.	Walks, hours.
Dog	7.9	17.9	5.0	5.2	5.5	6.0
"	3.7	8.3	2.5	6.5		
"	7.9	18.0	10.0	9.0	9.8	10.0
"	7.4	16.7	10.0	6.8	8.0	8.0
"	8.2	18.7	10.0	4.0	4.5	4.5
"	8.1	18.4	10.0	3.6	4.6	4.7
"			10.0			
"†	6.9	15.6	5.0	5.8	6.1	6.1
"†	7.4	16.8	0.0	...	9.5	9.5
"†	8.2	18.6	0.0	5.8	6.0	6.1
"†	4.8	10.8	0.0	...	...	13.0
"†	3.0	6.8	0.0	2.8	3.0	3.2
Rabbit	1.1	1.9	1.0	2.6	2.7	3.2
"	1.4	2.4	2.0	...	3.0	3.0
"	1.5	2.6	1.8	2.2	2.2	2.3
"	1.3	2.2	2.0	1.1	1.7	1.7
"	1.4	2.5	2.0			
"†			2.0	2.3	2.4	2.4
"†	1.5	2.7	0.0	3.0	3.1	3.3
"†	1.7	2.9	0.0	...	2.5	2.5
"†	1.2	2.1	0.0	...	2.1	2.2
"†	1.1	2.0	0.0	1.2	1.3	1.3

* Intraperitoneal injection.
† Control.

chloric acid was injected slowly into the external saphenous vein of the dog and into an ear vein of the rabbit in amounts varying from 2 to 10 cc.

TABLE 3.—PARALDEHYDE.*

Animal.	Dosage, gr.	Weight, kg.	HCl, N/10 cc.	Attempts to stand, hours.	Stands, hours.	Walks, hours.
Rabbit	52.5	2.7	2.0	1.0	5.9	6.1
"	46.5	2.4	2.0	4.9	5.9	6.3
"	51.0	2.6	2.0	4.3	6.0	6.1
"†	52.5	2.7	0.0	...	5.0	5.1
"†	48.8	2.5	0.0	3.0	4.1	4.7
"†	48.8	2.5	0.0	5.0	5.5	6.0

* Administered by stomach tube.
† Control.

In nembutal and paraldehyde anesthesia time was measured from the moment the prone animal showed evidences of general relaxation until the animal attempted to stand, stood, walked. In ether anesthesia, time was measured from the moment respiratory failure appeared imminent until the animal attempted to stand, stood, walked.

Groups of animals were anesthetized on the same day in an effort to duplicate all conditions. From the groups, random selection was made of control animals. The control animals were treated exactly like the others except that no hydrochloric acid was administered.

Results. Recovery from nembutal anesthesia or from ether anesthesia in the dog and rabbit was not hastened by the intravenous injection of N/10 hydrochloric acid.

Recovery from paraldehyde anesthesia in the rabbit was not hastened by the intravenous injection of N/10 hydrochloric acid.

Discussion. As in all work of this nature, individual differences in reaction were noted. It is felt, however, that a sufficiently large number of animals was used to justify the conclusions.

Immediately following the injection, the respiratory rate increased, as was expected. However, the recovery period was not shortened, but possibly lengthened.

The experiments were not continued further because no evidence whatsoever was found that the hydrochloric acid hastened the recovery from anesthesia.

Summary. Intravenous injections of N/10 hydrochloric acid do not hasten recovery of dog and rabbit from nembutal, ether or paraldehyde anesthesia. Apparently, such injections tend to lengthen the period of anesthesia.

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* It is not possible to compare the quantities of N/10 HCl used with those of MacGilvra or of Ellison and MacGilvra for these workers gave no detailed data. They mentioned that a total of 8.6 cc. of acid was injected in one patient but no other figures were given. No data were given of their animal experiments.

Landauer-'45
Bib No. 241

J. Exptl. Zoool. 98:65-77. 1945.

RUMPLESSNESS OF CHICKEN EMBRYOS PRODUCED BY THE INJECTION OF INSULIN AND OTHER CHEMICALS

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Incidental to other investigations it was observed that the injection of solutions of certain chemicals into unincubated chicken eggs led to the appearance of increased numbers of rumpless embryos and chicks. It was decided to make a systematic study of this problem, and the following is a first report on our work.

MATERIAL AND METHODS

The experiments were done chiefly with eggs from White Leghorn matings, but in one instance eggs from matings of Creeper fowl were used for a series of tests (insulin). Eggs were collected for periods of 1 week. During this time they were kept in an air-conditioned storage room. Eggs from the same mothers were distributed as evenly as possible into experimental and control groups. Prior to injecting the eggs they were allowed to stand in upright position for about 24 hours. After cleaning with alcohol a hole was drilled into the shell with a dental drill. This was done near the level of the short axis of the eggs. A tuberculin syringe was used for the injections, together with a no. 37, ¾-inch hypodermic needle. The amount of fluid injected was always 0.05 cc. The hole in the shell was sealed with Scotch tape. Treated and control eggs were incubated in the same forced-draft incubator.

The particular length of hypodermic needle used was chosen in order to be certain that the solutions would be injected into the yolk of the eggs. Was this actually accomplished and did the injected material not escape through the needle hole in the yolk membrane? Information in this respect was obtained by injecting eggs with 0.05 cc. of India ink, letting the eggs stand for 24 hours, hard-boiling them, and then opening them by a cut through their long axis. It was found that in each instance the major part of the ink was within the yolk, although traces were found along the path of the needle.

All embryos which had died between 14 and 18 days and between 18 and 22 days of incubation were examined for morphological abnormalities, as were the hatched chicks.

Standard errors are used throughout this report.

RESULTS

To start with, it was important to know if the damage done by insertion of the hypodermic needle or the injection of saline, which was in many instances used as solvent or diluent, has any effect on embryonic development. The results of such tests are given in table 1. Damage to the eggs, chiefly to the yolk membrane, is reflected in a greatly reduced percentage of hatched chicks. It is evident, however, that neither

TABLE 1

TREATMENT	NUMBER OF FERTILE EGGS	FERTILE EGGS HATCHED %	NUMBER OF EMBRYOS SURVIVING 17TH DAY	RUMPLESS %
Untreated control	253	76.7 ± 2.7	229	2.62 ± 1.05
Hypodermic needle inserted, but no injection	406	65.3 ± 2.4	337	4.45 ± 1.13
0.05 cc. saline injected	401	50.1 ± 2.5	241	2.70 ± 1.05

the insertion of a syringe needle nor the injection of saline into the egg yolk had any significant effect on the frequency with which rumplessness occurred. The same is true with regard to other developmental abnormalities.

Insulin

The insulin preparation used in our experiments was Iletin (Insulin, Lilly) in a concentration of 40 units per cubic centimeter. The amount injected was always 0.05 cc., i.e., 2 units. Table 2 shows the results of experiments with Leghorn eggs. Embryo mortality was greater than after the injection of saline, indicating a certain degree of toxicity of the insulin. The frequency of rumplessness among embryos which survived the seventeenth day of incubation was 41.6% as compared with 1.5% for the untreated controls, an increase of 2770%. The difference between treated and control lots was 40.1 ± 3.9%. Morphological abnormalities, other than rumplessness, were also more common in the insulin-treated material, but this difference was not statistically significant.

There are traces of zinc in Iletin, approximately 0.2 parts per million. Control experiments in which a much larger amount of zinc ions was injected (3.4γ $ZnCl_2$ per egg) did not result in a significant rise in the frequency of rumplessness.

The insulin preparation which we used contains 0.25% phenol and 1.6% glycerine and has a pH of between 2.5 and 3.5. In a search for possible sources of error a new series of experiments was undertaken in which, in addition to untreated controls, a third group of eggs was

TABLE 2

Results after injecting two units of insulin (Iletin) into Leghorn eggs.

DATE	TREATMENT	NUMBER OF FERTILE EGGS	FERTILE EGGS HATCHED %	NUMBER OF EMBRYOS SURVIVING 17TH DAY	RUMPLESS %	ABNORMALITIES OTHER THAN RUMPLESSNESS %
2/22/44	Iletin	93	20.4	39	46.2	10.3
	Control	112	75.0	104	0	0
3/7/44	Iletin	115	41.7	69	39.1	2.9
	Control	126	78.6	117	0.9	0.8
3/14/44	Iletin	118	40.7	65	41.5	1.5
	Control	132	71.2	111	3.6	1.8
Total	Iletin	326	35.3	173	41.6 ± 3.8	4.0 ± 1.5
	Control	370	74.9	332	1.5 ± 0.7	0.9 ± 0.5

injected with a solution which contained 0.25% phenol and 1.6% glycerine, and which was, with HCl, adjusted to a pH of 3. These experiments were done with eggs from matings of Creeper fowl. The results are given in table 3. It is evident that neither the phenol-glycerine admixtures, nor the hydrogen ion concentration were responsible for the high frequency of rumplessness which had been obtained in the earlier experiments. In fact, there is no significant difference between the phenol-glycerine group and the untreated controls. Insulin, on the other hand, again produced a high incidence of rumplessness. The difference in frequency of rumplessness among the insulin-injected group and the phenol-glycerine lot was 15.4 ± 2.2%, and that between the insulin group and the untreated controls amounted to 16.1 ± 2.2 %.

It is evident, however, that fewer cases of rumplessness occurred after injecting Creeper eggs than had been obtained in the earlier experiments with Leghorn eggs. The difference in average frequency of rumplessness in these two groups amounted to 24.5 ± 4.3% and is highly significant. It will be noted that the experiments with Creeper

eggs were performed later in the year than those with Leghorn eggs. Are we dealing with a seasonal difference in response? In order to test this question some Leghorn eggs were injected with Iletin on May 9, 1944. In this experiment sixty-three embryos survived the seventeenth day of development and 28.6% of these were rumpless. If this result is compared with the experiments performed with Creeper eggs on April 25 and May 2, it is found that the Leghorn eggs again produced the greater number of rumpless embryos. The difference amounts to 16.7 ± 6.2% and is significant. Further tests with Leghorn eggs were

TABLE 3

Results after injecting two units of insulin (Iletin) into Creeper eggs.

DATE	TREATMENT	NUMBER OF FERTILE EGGS	FERTILE EGGS HATCHED %	NUMBER OF SURVIVORS OF 17TH DAY	RUMPLESS %
4/4/44	Iletin	96	37.5	54	24.1
	Phenol-glycerine	94	35.1	61	0
	Untreated	95	56.8	70	0
4/11/44	Iletin	98	31.6	55	23.6
	Phenol-glycerine	93	51.6	66	3.0
	Untreated	97	43.3	64	0
4/18/44	Iletin	94	50.0	65	18.5
	Phenol-glycerine	93	50.5	67	3.0
	Untreated	98	46.9	70	1.5
4/25/44	Iletin	132	42.4	84	11.9
	Phenol-glycerine	125	42.4	84	1.2
	Untreated	150	50.7	98	1.0
5/2/44	Iletin	137	28.5	76	11.8
	Phenol-glycerine	136	41.2	80	1.3
	Untreated	140	50.7	92	2.2
Total	Iletin	557	37.5 ± 2.1	334	17.1 ± 2.1
	Phenol-glycerine	541	43.8 ± 2.1	358	1.7 ± 0.7
	Untreated	580	49.8 ± 2.1	394	1.0 ± 0.5

conducted between June 20 and July 11. In these experiments a total of 455 embryos and chicks survived the seventeenth day of incubation and there were 112 or 24.6 ± 2.0% cases of rumplessness. This was again a significant excess over the response shown by Creeper eggs during the latter part of the season or even, in fact, throughout the whole experimental period. Hence it may be concluded that, as far as the production of rumplessness is concerned, the eggs of the particular Leghorn and Creeper stocks which were used in our work differed in degree of response to insulin. This conclusion is supported by observa-

tions on embryonic mortality. In comparing the two stocks in this respect, allowance must be made for the homozygous lethal effect of the Creeper factor, which reduces hatches by about 25%. If this is taken into account, it can be seen that injection of insulin into Creeper eggs reduces hatching to a much lesser degree than does the same treatment of Leghorn eggs; in other words, the insulin has a less toxic effect on eggs of the Creeper stock than on those of the Leghorn stock. It cannot be determined with certainty from our material whether the less drastic effect of insulin on Creeper eggs is due to the Creeper factor itself or to residual heredity. Among the fifty-seven rumpless embryos and chicks of this material thirty-two were Creeper and twenty-five normal, with an expectation of two Creepers to one normal. There is a deficiency of Creepers, as would be expected if the Creeper factor provided protection against the interference with normal rump formation, but the data are not good enough to establish this point.

The conclusion that the eggs of our Creeper stock tend to react less extremely to insulin is supported further by the more common occurrence of intermediate rumpless cases in this material. Among ninety rumpless embryos and chicks from the Leghorn experiments all but five (5.6%) were completely rumpless. Among fifty-seven rumpless embryos and chicks from the Creeper stock, on the other hand, ten or 17.5% showed stages intermediate between complete rumplessness and normal conformation.

The differences in response to insulin, obtained with Leghorn and Creeper eggs, are not entirely accounted for, however, by genetic dissimilaritiy of the stocks. Another interesting feature of our observations is the gradual decline in the percentage of rumpless embryos produced in successive experiments. In the Leghorn eggs 46.2% rumpless embryos and chicks were found in the test of February 22, but only 28.6% in that of May 9 and an average of 24.6% in four tests made between June 20 and July 11. In the Creeper eggs the percentage of rumpless embryos fell from an initial high of 24.1% (April 4) to 11.8% (May 2). Chemical changes in the insulin can be ruled out as a possible cause since the supply of Iletin was replenished twice in the course of the experiments. It is most likely that the observed changes in response represent a seasonal gradient due to gradually increasing environmental temperatures and consequent changes in the developmental stages of the eggs at the time the injections were made. This problem will be the subject of further studies.

The frequency of morphological abnormalities other than rumplessness was 3.0 ± 0.9% in the insulin-injected Creeper eggs, 1.1 ± 0.6% in

the phenol-glycerine controls, and 0.5 % 0.4% in the untreated controls. As in the Leghorn material, there is a suggestion that the incidence of all abnormalities is increased by the influence of insulin, but the differences are not significant.

The records of the insulin-injected Leghorn eggs have been analysed with regard to homogeneity of response among the mothers which produced the eggs. The data were arranged in an R × 2 table. A χ^2 of 685.8 was found, with 104 degrees of freedom. Since the 1% point is about 6334 and the 5% point is 253, the data definitely suggest heterogeneity i.e., the mother's genetic constitution appears to influence the chances whether or not her eggs will respond to insulin injection with the production of rumplessness.

TABLE 4

Results of the injection of cystine. Experiments with Leghorn eggs.

TREATMENT	NUMBER OF FERTILE EGGS	FERTILE EGGS HATCHED %	NUMBER OF SURVIVORS OF 17TH DAY	RUMPLESS %
0.2 mg. cystine per cc.	181	55.8	141	6.4 ± 2.1
46.4 mg. cystine per cc.	194	57.7	158	6.3 ± 1.9
Total	375	56.8	229	6.3 ± 1.4

In earlier experiments it had been found that the injection of cysteine hydrochloride led to a considerable rise in the frequency with which rumplessness occurred. The eggs were injected with 0.05 cc. of a solution of 100 mg. per cubic centimeter of cysteine hydrochloride in saline. Among 111 embryos which survived the seventeenth day 12.6 ± 3.2% were rumpless. The difference from the untreated control eggs was 10.1 ± 3.3%. This result raised the question whether the effect of insulin is possibly due to its cystine content. Experiments were made to test this. Leghorn eggs were used. Cystine was dissolved in saline by addition of just enough HCl to obtain solution. In a first series the concentration of cystine was 0.2 mg. per cubic centimeter. By injection of 0.05 cc. of this solution into each egg, the amount of cystine was equivalent to that of two units of insulin, the quantity which was used in our experiments. In a second series the concentration of cystine was 232 times that of the first series (46.4 mg. per cc.). The data of these experiments are shown in table 4. The results were nearly identical in the two tests, in spite of the great difference in cystine concentration. The pH of both solutions was about 2. As compared with untreated controls, cystine injection produced a significant increase in the

occurrence of rumplessness. As compared with the insulin experiments, the results demonstrate that cystine alone is not responsible for the effect of insulin.

Experiments were made with one other amino acid, viz., glutamic acid hydrochloride. Both l- and dl-glutamic acid hydrochloride were used. We injected 0.05 cc. of a solution of 547 mg. per cubic centimeter of l- or dl-glutamic acid hydrochloride dissolved in saline. The results of these tests are assembled in table 5. The increase in frequency of rumplessness, as compared with the untreated controls, was 3.1 ± 1.0% for the l-glutamic acid hydrochloride and 6.8 ± 1.3% for the dl-glutamic acid hydrochloride. Both forms of glutamic acid hydrochloride produce

TABLE 5

Results of the injection of l- or dl-glutamic acid hydrochloride. Experiments with Leghorn eggs.

TREATMENT	NUMBER OF FERTILE EGGS	FERTILE EGGS HATCHED %	NUMBER OF SURVIVORS OF 17TH DAY	RUMPLESS %
l-glutamic acid hydrochloride	776	48.2	345	4.6 ± 0.9
dl-glutamic acid hydrochloride	772	48.6	356	8.3 ± 1.2
Untreated	822	68.7	726	1.5 ± 0.5

a significant rise in the frequency of rumplessness. The data also suggest the possibility that dl-glutamic acid hydrochloride is more effective than l-glutamic acid hydrochloride, the difference amounting to 3.68 ± 1.47%. On the other hand, there is certainly no suggestion in these data that the glutamic acid of insulin plays an important role in the insulin effect on rump development. No significant increase in incidence of morphological abnormalities other than rumplessness was produced by either of the glutamic acid hydrochlorides.

Tests with other substances

A series of experiments was run with l-, dl- and d-malic acid. The l- and dl-malic acids were commercial C.P. products. The d-malic acid was prepared for our experiments by Prof. A. W. Ingersoll. We injected 0.05 cc. of solutions containing 20 mg. per cubic centimeter of the three types, respectively, of malic acid. The results are shown in table 6. As judged by embryonic mortality, none of the three preparations had a pronounced, if any, toxic effect. Injection of d-malic acid had no significant influence on the occurrence of rumplessness. In the

group which was injected with dl-malic acid the frequency of rumplessness among embryos and chicks was 2.6 ± 1.1% greater than in the untreated controls. This suggests a significant increase. Such a rise in the production of rumplessness definitely followed the injection of l-malic acid, the difference from the control material being 3.0 ± 1.1%. If the results with dl- and l-malic acid are combined, it is found that there is an increase in rumpless frequency of 2.8 ± 0.9%. Abnormalities other than rumplessness occurred with the following frequencies: 0.2% in the controls, 1.5% each in the l- and d-malic acid groups, and

TABLE 6

Results of the injection of l-, dl-, and d-malic acid. Experiments with Leghorn eggs.

TREATMENT	NUMBER OF FERTILE EGGS	FERTILE EGGS HATCHED %	NUMBER OF SURVIVORS OF 17TH DAY	RUMPLESS %
l-malic	523	58.7	413	4.3 ± 1.0
dl-malic	513	57.3	401	3.9 ± 1.0
d-malic	519	59.0	401	2.0 ± 0.7
Untreated	526	69.4	457	1.3 ± 0.5

TABLE 7

Results of the injection of thioglycollic acid, hydrochloric acid and saline. The thioglycollic acid and saline were 0.2 molar with a pH of 7; the hydrochloric acid had a pH of about 1. Leghorn eggs.

TREATMENT	NUMBER OF FERTILE EGGS	FERTILE EGGS HATCHED %	NUMBER OF SURVIVORS OF 17TH DAY	RUMPLESS %
Thioglycollic acid	648	41.4	374	6.7 ± 1.3
Hydrochloric acid	1141	60.0	708	3.5 ± 0.6
Sodium chloride	864	46.5	537	2.2 ± 0.6
Untreated	1072	73.5	930	1.9 ± 0.5

2.2% in the series injected with dl-malic acid. The differences are suggestive of some effect in all groups, but are not significant.

In another series of experiments we compared the effects of thioglycollic acid, hydrochloric acid and a sodium chloride solution. The thioglycollic acid and sodium chloride solutions were 0.2 molar and had a pH of 7. The hydrochloric acid was adjusted to the same pH (about 1) as the solutions of glutamic acid hydrochloride which were discussed above. The molarity of the hydrochloric acid was the same as that of the least concentrated cysteine hydrochloride solution used in our experiments (20 mg. cysteine hydrochloride per cc.). The amount injected of each solution was 0.05 cc. per egg. The results are shown in table 7.

As compared with the untreated controls, thioglycollic acid accounted for a significant rise in the frequency of rumplessness, the difference between the two groups amounting to $4.8 \pm 1.3\%$. The treatment with hydrochloric acid and sodium chloride, on the other hand, had no effect in this respect. It should be noted, however, that hydrochloric acid, if injected in concentrated form (0.05 cc.), probably does produce a significant rise in production of rumpless embryos. In such a test $3.8 \pm 1.1\%$ rumpless embryos were found as against $0.8 \pm 0.5\%$ among the untreated controls, the difference between the two groups amounting to $3.0 \pm 1.2\%$. No significant increase in the frequency of malformations other than rumplessness was observed in any of these tests.

DISCUSSION

The outstanding result of our experiments is the high percentage of rumplessness produced by the injection of insulin. This appears to be the first recorded instance of a morphogenetic effect of insulin.

The morphological appearance of the distal end of the spine in insulin-induced rumplessness does, in most cases, resemble the sporadically occurring cases. Those rumpless embryos, in the insulin experiments, which died shortly before hatching time or which at the end of the incubation period were found alive within the shell were nearly all completely rumpless. In many of these embryos some of the synsacrocaudal vertebrae were missing in addition to the free tail vertebrae. In certain of the most extreme cases the posterior end of the body was edematous. Among the rumpless chicks which hatched, on the other hand, there were some which had a partial tail skeleton. In this respect the material differs from that of sporadic rumplessness since in the latter no intermediate forms have ever been observed.

In earlier experiments it was shown that rumplessness can be produced by mechanical shaking of eggs (Landauer and Baumann, '43). As in these shaking experiments, it was found after insulin treatment that the genetic constitution of the mother plays a role in the extent of response obtained under experimental conditions. It does not seem likely that the genetic factors which protect embryos from becoming rumpless on account of shaking of the eggs or because of the injection of insulin are the same ones.

In the case of shaking the evidence suggested that the results are brought about by injury to the blastoderm. It was shown that these mechanical forces not only raise the incidence of rumplessness, but also, and to a similar extent, that of all other commonly occurring malformations. This is not so in the case of insulin treatment. It is true

that the experiments suggest a slight (though not statistically significant) increase in the frequency of all types of abnormalities. This may be due to mechanical disturbances produced by injection per se. At any rate, it is obvious that insulin has a selective effect on rump development. It seems reasonable to assume that the selective response of the caudal part of the vertebral column is in some way related to developmental peculiarities of this region. Such perculiarities do actually exist. Degeneration of presumptive tail tissue plays a role in normal development of the tail and it is also the principal feature in the origin of dominant rumplessness (Zwilling, '42). In later stages cartilage differentiation in the tail lags behind that of other parts of the skeleton (Blinov, '38). These developmental features are probably in some way related to the effect which insulin has on formation of the tail.

How this effect is brought about cannot at present be determined with certainty, but it may be well to discuss some aspects of the problem. The physiological properties of insulin suggest the possibility that an interference with the embryo's carbohydrate metabolism may occur after its injection. But even if this assumption is correct, it remains entirely unknown why development of the tail should be affected selectively. Corda ('32) found that the injection of insulin into pregnant rabbits was followed by the abortion of undersized fetuses and the birth of small young. The results were ascribed to interference with the carbohydrate exchange between mother and fetuses, but the author envisioned also the possibility of a more direct action of insulin on the fetus. There were no teratological effects. Gregg and Pomerat ('42), working with developing Rana pipiens embryos, found that insulin was inhibiting and toxic above a concentration of 0.02%, but no effect of any kind was observed at lower concentrations. These results do not shed any light on our observations.

The fact that two of the amino acids which contribute most to the composition of insulin (cystine and glutamic acid), if injected by themselves, do not produce a rise in the occurrence of rumplessness comparable to that obtained with insulin, does not exclude the possibility that the rumpless effect may be due to still other amino acids or the combined action of more than one of them. This must be settled by further experiments.

It is of interest to point out that some of our most significant results were obtained after the injection of substances containing sulphur (insulin, cysteine, cystine, thioglycollic acid). The possible role of sulphur in the production of rumplessness requires further investigation. Our data show, however, that other substances, e.g., d-glutamic

acid hydrochloride, may lead to similar results. Ancel and Lallemand ('42) reported that treatment of early chicken embryos with certain chemicals resulted in rumplessness, but gave no further details.

Our observations do not support the conclusion that acidification per se is instrumental in diverting development in the direction of rumplessness.

It is known that differences in toxicity exist between the optical isomeres of certain substances (Cushny, '26; Gause, '41). If the production of rumplessness were a consequence of toxic action, then our observations on glutamic and malic acid could be taken as another instance of this kind. The isomeres which do not occur naturally are, in general, the ones which are more toxic. In the case of glutamic acid hydrochloride no differences in embryonic mortality existed after the injection of the l- and dl- forms, but the dl-glutamic acid hydrochloride produced nearly twice as many cases of rumplessness as did the l-glutamic acid hydrochloride. As judged by embryonic mortality there was also no difference in the effect produced by the isomeres of malic acid, but here it was the naturally occurring form (l-malic) which was most effective in bringing about the development of rumplessness. It is clear, therefore, that our results cannot be accounted for by differences in toxicity, but must be due to specific reactions of the embryo to particular isomeres.

Inhibitory and toxic effects of certain amino acids (tyrosine, phenylaline, cysteine) on embryonic development of a beetle (Bruchus quadrimaculatus Fabricius) were reported by Brauer ('42). This author also found selective susceptibility of certain embryonic regions and it must be such a selective response which accounts for the origin of rumplessness in our experiments.

Our observations demonstrate that rumplessness can be produced by a variety of chemical substances. In earlier work it was shown that rumplessness can also be induced by mechanical forces. The possible relation of these phenocopies to the developmental aspects of hereditary rumplessness cannot be discussed until a study of the embryonic development of experimentally-produced rumplessness has been made. In a general way, however, conclusions at which Grüneberg ('43, p. 243) arrived in a discussion of x-ray induced phenocopies in mice may apply also to hereditary rumplessness. He says: "One cannot help getting the impression that these structural changes, at any rate, are rather unspecific results of gene action. Some more fundamental change in development seems to upset those developmental processes, which at a given time happen to be in a critical and hence susceptible stage." With

regard to details of developmental events we do not know at present if any experimentally-produced phenocopy is truly analogous to its hereditary counterpart. Yet, even if this were not true for the present material, it would still remain a fact of considerable interest that, as in the case of the mutations for rumplessness and their modifiers, insulin can bring about either complete rumplessness or varied modifications intermediate between this condition and a normal tail.

SUMMARY

Experiments in which various chemical substances were injected into the yolk of eggs prior to incubation, had the following results.

After the injection of two units of zinc-free Iletin (Insulin, Lilly) into eggs of White Leghorn fowl 41.6 ± 3.8% of all embryos surviving the seventeenth day were rumpless. Among the controls there were only 1.5 ± 0.7% of rumpless embryos and chicks.

Similar experiments with eggs of Creeper fowl resulted in 17.1 ± 2.1% rumpless embryos with 1.0 ± 0.5% rumplessness among the controls.

The results are highly significant in both stocks. The difference between the stocks is due in part to variations in response on account of genetic dissimilarity and in part to seasonal influences. Significant heterogeneity in response of the eggs to insulin treatment was found among the hens involved in the experiment with Leghorn fowl.

Significant increases in the frequency with which rumplessness occurred were obtained also after the injection of cysteine hydrochloride, cystine, dl-glutamic acid hydrochloride, thioglycollic acid and l-malic acid. The effect of l-glutamic acid hydrochloride was much less pronounced than that of the dl- form; d-malic acid had no effect. Concentrated hydrochloric acid probably produced some increase in rumpless frequency, but this was not true for more dilute hydrochloric acid (pH 1). Injection of saline or mere insertion of a hypodermic needle gave no results.

None of the experiments led to a significant rise in abnormalities other than rumplessness; not, at any rate, among the late embryonic stages which we examined.

ACKNOWLEDGMENTS

The author is under great obligation to Prof. A. W. Ingersoll, Vanderbilt University, Nashville, Tennessee for preparing the d-malic acid used in our experiments and to Dr. F. B. Peck and the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana for pro-

viding the Iletin (Insulin, Lilly) as well as for information concerning this product.

The author is especially grateful to Dr. Heinrich Waelsch, New York State Psychiatric Institute and Hospital, New York City for advice and help in many ways.

The author is much obliged to Dr. E. M. Bailey, Department of Analytical Chemistry, Connecticut Agricultural Experiment Station, New Haven for a zinc determination in a sample of Iletin (Insulin, Lilly).

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Canad. M. A. J.
Aug. 15, 1955, Vol. 73

Case Reports

ACUTE ACID ŒSOPHAGITIS

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ACID BURNS occur much less frequently than those caused by caustics; they are mostly encountered in adults, and result not only from accident but also from suicidal intent. The majority of chemical burns of the œsophagus are second-degree burns in which necrosis of mucous membrane and muscular layer takes place. The necrotic tissue sloughs and granulations form. Unless the formation of scar tissue can be prevented or controlled, a stricture of the œsophagus will develop.

General management of these corrosive acid burns may be divided into three obvious categories, according to the following writers: Belinoff, Jackson and Jackson, Barretto, Holinger, Negus, Plummer, Tucker, Vinson, Lemariey, Krey, Cleland and Leary.

The first type of treatment includes: (a) Administration of neutralizing substances such as olive oil, 5% sodium bicarbonate solution associated with 2 g. of bismuth subcarbonate, every four hours; magnesium oxide and bismuth subnitrate may be substituted. The use of small quantities of diluted alkalis at frequent intervals seems indicated for the first five days. (b) Supporting measures to prevent shock, dehydration and electrolyte imbalance. (c) Use of sedatives and atropine against pain and hypersalivation, which requires frequent aspiration. (d) Lavage of the stomach, which should be attempted only if the Levine tube can be introduced easily, without danger of perforation. This kind of lavage is not a feasible manœuvre in most cases of acid burn.

The second type of procedure is for the prevention of stricture: (a) Immediate threading, by having the patient swallow a string or a Levine tube when it is possible. (b) Early jejunostomy rather than gastrostomy. The former operation is suggested as a routine procedure in cases of severe acid burns, but involves special feedings; the latter may be contraindicated in view of damage to the stomach; the gastrostomy stoma may be useful for feeding, drainage and future dilatation. (c) Early bouginage. There are two schools of thought as to the time such treatment should be started. Salzer and his followers advocate the passage of soft rubber bougies between the second and sixth days after ingestion of the corrosive. A second group of authors (Belinoff, Plummer, the Jacksons and Moersch) suggest bouginage after two weeks, but under direct vision or along a string swallowed as a guide for threading olive-tipped bougies for subsequent dilatation. Œsophagoscopy or dilatation with the mercury-filled Hurst bougie should never be performed during the early stages.

The third type of procedure is for treatment of already formed stenosis. Here the modified Tucker retrograde dilatation through a gastrostomy opening, as advocated by Barretto, is a much more efficient technique for tight strictures: it consists of passing Plummer metal bougies along a continuous string from above downward, the string being grasped and fixed firmly at the gastrostomy opening. Harvey and Negus replace the Plummer bougies by plastic graduated bobbins or moles. In carrying out this procedure in cases of complete atresia, fluoroscopy in two planes is usually necessary. These manœuvres are continued until an œsophagoscope can be passed along the course of the œsophagus with ease, at which time the string is removed; the gastrostomy is allowed to close, and subsequent dilatations are made with Hurst bougies or the œsophagoscope.

The above summarizes the commonly accepted methods of treatment in acid burns of the œsophagus. For comparison, I report the management of a hydrochloric acid burn of the mouth, œsophagus and stomach.

L.G., a psychopathic man, 37 years of age, swallowed about 8 oz. of commercial muriatic acid in a suicidal gesture on November 8, 1953. The next morning at 9:30 his wife came to our clinic with the patient. At this time a slight white swelling was noticed on the soft palate; there was much more œdema at the vestibule of the larynx and pyriform sinus. At 10:30 a.m. the patch of œdema became whiter and was growing to the extent of necessitating emergency tracheotomy; immediate supportive measures and sedatives were given. On account of the impossibility of swallowing, no attempt was made to have the patient swallow a string or Levine tube. Even radiography was postponed. Œsophagoscopy was considered contraindicated.

On November 11, the third day, complete desquamation of the necrotic membranes in the mouth was noticed; the severe œdema of the laryngo-pharynx had diminished and necrotic membranes were still present there. When an attempt was made to have the patient swallow some water, the liquid passed through the tracheal cannula. The general condition did not allow the administration of cortisone or ACTH.

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On November 14, a gastrostomy was performed by Dr. R. Decarie. His findings were as follows. "On the anterior wall of the stomach is seen a necrotic ulcer, near the fundus; it is 2 inches wide and ½ inch deep; the bottom of this ulcer is very thin, sloughed, and dark green. The gastric mucosa looks necrotic, with dark exudate." On the day following the operation, temperature was 102° F.; deep toxicity of the patient was noticed and necrosis of the stoma was taking place. On November 21, phlegmonous reaction of the gastrostomy stoma with necrosis of the surrounding abdominal wall layers was present. Periodic irrigation with mild Dakin solution was carried out.

On November 26 and 28 great improvement in the surgical wound appearance and the general condition was noticed. The patient began to swallow and drink water and even sit in his chair. During these few days, vomiting of yellowish material occurred once a day but to our knowledge there was no passage of slough. On December 4 the mouth and the laryngo-pharynx looked normal. Decannulation was carried out. On December 7 radiographs showed a normal œsophagus; the stomach presented incomplete cicatricial stricture at its middle third, the pylorus being normal.

Herniation of the stomach through the abdominal wound did not allow œsophagoscopy until December 30. The lumen and mucous membrane looked normal along the whole course of the œsophagus.

Among the most important laboratory findings we should mention: red cell count not lower than 3,300,000 on December 10; white cell count up to 22,000 on November 26; Hb not lower than 68% on November 26; blood potassium down to 5.9 mg. per 100 ml. against 16 to 24 mg. normal, with signs of acute nephritis. Temperature went up to 104° F. on November 15 and 20, i.e. after the gastrostomy.

The patient left hospital in fairly good condition. On January 8, 1954, he was readmitted for electro-convulsive therapy. On the morning of August 25, 1954, the patient made a second attempt at suicide by ingesting corrosive liniment (ammonia combined with methyl salicylate), but without sequelæ. He has since been sent to a hospital for mental diseases.

COMMENTS

Cleland reported a case in which the slough was expelled on the ninth day after swallowing hydrochloric acid.

In our case, no dilatation procedure was used, but moderate stricture of the gastric cavity resulted after the gastrostomy.

It may be that, by allowing better drainage, gastrostomy prevented more serious scar stricture than a jejunostomy would have done.

From Cleland's report and our own case, it seems that hydrochloric acid burns are less severe than nitric or sulphuric acid burns of the œsophagus.

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MacGilvra-'36
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J. Am. Dent. Assoc. 23:668-674. 1936.

INTRAVENOUS METHOD OF REVERSING THE PROCESS OF ANESTHESIA*

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THE subject of this paper would be more appropriately presented under the title "Further studies in the use of intravenous injections of twentieth normal hydrochloric acid for the threefold purpose of: (1) lowering thresholds of stimulation, (2) stimulating respiration and (3) elevating blood pressure in cases of postoperative collapse following general anesthesia."

Subsequently to the presentation of a preliminary report[1] on our verification of and observations on the discovery of Pearl L. Moorman, medical technician of the Duncan Laboratories, Joplin, Mo., and the sequences of its successful clinical

*Read at the Sixteenth Annual Meeting of the American Society of Oral Surgeons and Exodontists, St. Paul, Minn., Aug. 5, 1935.

1. MacGilvra, W. V., and Ellison, Alfred: Palinesthesia: Preliminary Report on Effects of Recall Acid as Means of Recovery and Restoration to Sensibility, unpublished data. MacGilvra, W. V.: Story of Palinesthesia: Effects of Recall Acid as Means of Recovery to Sensibility, Harvard D. Rec., 8: 1-4 (Jan. 31) 1934.

use to correct grave symptoms in a case of overdose of basal anesthesia, wide publicity was given, which in turn provoked lively discussion, led chiefly by Shambaugh and Boggs.[2]

A brief review of the first clinical case may show cause for our early enthusiastic hope that a promising remedy, for emergency use, was somewhere in sight.

This case furnished its own control, the patient undergoing operation twice in thirty-five days, basal anesthesia tribromethynol (avertin) being used on both occasions. The status of health of the patient on the dates of operation was far from being identical.

The condition of this patient on the date of the first operation may be compared to that of a normal adult having an acute appendicitis. At the time of the second operation, after several weeks of general peritonitis complicated by cellulitis of the abdominal wall, the status of health was very different.

From an anesthetic standpoint, the case had, in thirty days, changed from a good to a poor anesthetic risk.

By virtue of a series of circumstances and notwithstanding the great change in the anesthetic risk, on the occasion of the second operation, the patient received a dose of avertin on the basis of his weight at the time of admission nearly five weeks previously, and not on the basis of his actual weight at the time. Therefore, in all probability, he was given a dose somewhat in excess of 100 mg. per kilogram of body weight; whereas, he had received but 90 mg. per kilogram before appendectomy, the first operation. The second operation, incidentally, was merely an incision in the abdominal wall to increase drainage.

2. Shambaugh, Philip, and Boggs, Robert: Effect of Intravenous Hydrochloric Acid upon Narcosis in Dogs, Proc. Soc. Exper. Biol. & Med., 31:2 (March 31) 1934.

Embarrassing as it is to admit the error, the patient was a victim of an ill-advised dose.

At operation, the patient, as might be expected, was moribund. Nor was this my judgment alone, the opinion being shared by every member of the staff present.

The patient presented the following symptoms: deep cyanosis, clear air ways, rapid, shallow respiration, barely perceptible tidal volume, high pulse rate of feeble quality, low blood pressure, very low pulse pressure and complete lack of muscle tone.

DATA ON CASE 1

First Operation.—June 20, 1933, appendectomy was performed on the patient, a man, aged 50, weight 142 pounds, height 5 feet 4 inches. A basal dose of 90 mg. per kilogram of body weight was given: i.e., avertin, 5.8 c.c.; water, 232 c.c. The preanesthetic pulse was 80. A preliminary hypnotic of morphine, one-sixth grain and atropine, one-one hundred and fiftieth grain, was given. Gas-oxygen anesthesia was superimposed. The duration of the operation was one hour and ten minutes; total duration of unconsciousness, two hours and thirty minutes.

Second Operation.—July 25, 1933, an incision was made to increase drainage. The basal dose was 95 mg.: avertin, 6.2 c.c.; water, 247 c.c. The preanesthetic pulse was 120. There was no preliminary hypnotic and no anesthetic superimposed. The duration of the operation was fifteen minutes; total duration of unconsciousness, one hour and seven minutes.

After the second operation, 8.6 c.c. of tenth normal hydrochloric acid was given intravenously.

The difference in orientation time was one hour and twenty-three minutes.

Following the injection of the first few drops of hydrochloric acid, there was an immediate increase in tidal volume and the chain of returning reflexes began in eight minutes, continuing steadily until, at the ex-

piration of thirty-five minutes, the patient was fully conscious, a thing unusual after so large a basal dose of avertin.

So satisfactory was the outcome of this first attempt, so far reaching were the results, and so promising appeared the future in similar attempts, that it was decided to present the subject to Leroy M. S. Miner, dean of the Harvard University Dental School, and to enlist the aid of the school in continuing research to bring out the facts surrounding this phenomenon. This aid sought from Dr. Miner was immediately given, and, through him, the cooperation of the Harvard Medical School was enlisted to make a preliminary survey of the problem. In connection with this research, a committee was formed of the faculties of the Harvard Medical and Dental schools.

It was my privilege to share in the work of a subcommittee which conducted the preliminary survey of the problem, a summary of whose work disclosed the facts tabulated as follows:

Cardiac depression, central:

Dog—Blood pressure level an important factor where blood pressure essentially the same. Depression of heart is increased after administration of hydrochloric acid, 10-20 per cent.

Cat—Same as dog.

Blood pressure:

Dog—Not raised by administration of hydrochloric acid (?) Anesthesia.

Cat—Raised from 100 to 128 mm. of mercury.

Cat—No artificial respiration; ether.

Vagal response:

Slightly greater (5-10 per cent) after administration of hydrochloric acid.

Peripheral vagal response:

Also (5-10 per cent).

Femoral blood pressure:

Raised—response greater (10-20 per cent) after administration of hydrochloric acid.

Blood pressure:

Steady, slow rise after administration of hydrochloric acid.

Cortex:

Cat (dial) threshold, before administration of hydrochloric acid: 8:5-9; after: 8-8:5.

Monkey (ether) face: before: 9.0-9.5; after: 9.5-10. Arm: before: 9.5-10; after: 10. Leg: before: 8.-8.5; after: 8.5-9.

Spinal reflexes (Cor. ext.):

2 Cats (decerebrate): Definite lowering of threshold and increased response (30 per cent).

Respiratory center:

100-300 per cent.

Cardiac depression—peripheral:

Dog (ether): Blood pressure level most important factor—depression increased after administration of hydrochloric acid, pulse and blood pressure particularly increased.

It is evident from the foregoing that these experiments, while roughly quantitative, show a lowering of the threshold for electrical stimulation, in numerous portions of the nervous system, tested on several species of animals under various types of narcosis. The respiratory center was strongly affected, the tidal volume being greatly increased, while the blood pressure increased gradually and was sustained.

In contrast with the expriments reported by Shambaugh and Boggs,[2] in whose experiments the animals were merely anesthetized and not operated on, it may be pointed out that, in clinical practice, the patients here studied are human beings all of whom have undergone surgical operations, and in whom the stimulation of surgical trauma is present at the time of acid injection. Even though the threshold for stimulation is elevated by reason of anesthesia and shock, the lowering of the threshold by virtue of the acid effect permits the stimulus present free access to tracks, synapses

and centers previously incapable of transmission and lacking in sufficient irritability to react to stimulation. That synapses are affected by intravenous injection of hydrochloric acid was suggested in a casual experiment not scheduled on our program in which a co-worker in the same laboratory engaged on a wholly unrelated problem had paralyzed the synapses in a cat by using nicotine. After completing his own work, he turned over the animal to us. The acid was injected and the paralyzed synapses became immediately functional. Nicotine was again injected and the synapses became paralyzed, and again acid established their integrity.

Results of the foregoing experiments would tend to throw doubt on any conclusions that the intravenous injection of dilute hydrochloric acid is without effect.

The slides shown illustrated graphically the condition of the patient previous to, and after, acid injection, all in this series receiving hydrochloric acid in a twentieth normal concentration. The volume of acid injected varied in individual cases, each patient receiving only sufficient to produce a favorable change in his condition. The effect produced depends less on the speed of injection than on the volume injected. Elapsed time during injection has varied from one to twenty minutes.

Some of these patients received other forms of stimulation, coincident with the intravenous injection of acid, and this may be explained as necessary since it is admitted that our work is purely experimental and we could not in justice to the patient escape the use of recognized emergency aids. It is felt that because of the effect of intravenous injection of the acid, more pronounced results were obtained from the other stimulants used.

As far as general anesthesia is concerned, the members of this society are perhaps more largely interested in nitrous oxide-oxygen, than in ether, and basal anesthetic agents, though all have a proper place in your practice, and you would doubtless be interested to learn of what use intravenous injection of hydrochloric acid might be in the case of gas anesthesia.

As yet, we have had no opportunity to use this method in any case of overdose with the anesthetic gases. Experimental work with rabbits shows spectacular results, even when the animal is anesthetized to the point of respiratory failure. It must be admitted that this is true even without use of the acid, if one is successful in reestablishing respiration. The point is that injection of the acid into the circulating blood not only lowers the threshold for the respiratory center to receive stimulation, but, by its reaction with the bicarbonates of the plasma, enriches the blood with an increased carbon dioxide, the specific stimulant desired. The proper time for its use is early in the period of respiratory cessation and before heart failure. No time should be lost in giving it, if respiration by the usual means cannot be promptly reestablished.

In the event of asphyxial respiratory failure from overdose of an anesthetic gas in a human being, the same results may be predicted, and even though the respiratory function were to be reestablished and continued for so short a time as five minutes, the increase in tidal volume would eliminate practically all of the anesthetic agent, thus removing the hazard.

The fate of the dilute solution of hydrochloric acid injected into the blood stream is explained by Wright,[3] as follows:

I. Physico-Chemical Reactions. 1. When any non-volatile acid like lactic, phosphoric,

3. Wright, Samson: Applied Physiology, Ed. 4, London: Oxford University Press, 1932.

or sulphuric acid enters the blood stream, it at once interacts with the bicarbonate present in the plasma as follows:

(i) HL $NaHCO_3$ NaL H_2CO_3
(Lactic acid) (Sodium lactate)

In the case of lactic acid, sodium lactate and carbonic acid are formed. The advantage of this reaction to the body is clear. Lactic acid is a strong acid, which means an acid which dissociates freely and gives rise to many H ions, and thus makes any solution in which it is present very acid: it is also a non-volatile acid which is excreted slowly in the urine. By the interaction with sodium bicarbonate it is converted into a neutral salt and H_2CO_3 is liberated. Now H_2CO_3 is a much weaker acid, giving rise to fewer H ions; furthermore, it is a volatile acid which is readily eliminated in the lungs. Sodium bicarbonate has therefore acted as a buffer substance; it has caused H ions to be "mopped up," fewer are left in the solution, and reaction of the blood has altered to a much slighter extent than otherwise would have been the case. This is why the term "alkali reserve" was introduced to describe the bicarbonate of the blood.

It follows that as long as $NaHCO_3$ is present in the plasma, no acid stronger than carbonic can exist in the blood as such.

2. But the acid formed in largest amounts in the body is H_2CO_3 itself, which of course cannot be dealt with by $NaHCO_3$. H_2CO_3 is dealt with in the following way:

(ii) H_2CO_3 NaCL $NaHCO_3$ HCL
(iii) HCL KHb KCL HHb
(Potassium salt of reduced hemoglobin.)

H_2CO_3 interacts with the sodium chloride of the plasma to form sodium bicarbonate and hydrochloric acid. On the face of it this reaction seems both meaningless and impossible. Instead of the weak acid H_2CO_3 we have formed the stronger acid HCl, so the H ion concentration of the solution ought to rise still higher. Again, a weak acid like H_2CO_3 cannot displace chloride from its union with Na in NaCl. The explanation is this. An equation like (ii) above can act in either direction if the product of the reaction that would tend to drive the process the opposite way is at once removed. In other words, the equation will go from left to right if the HCl formed is at once removed from the plasma. This actually happens. If blood is exposed to increased tensions of CO_2, the amount of $HaHCO_3$ in the plasma increases and the chloride diminishes in the plasma and increases in the interior of the red blood corpuscles. The HCl resulting from equation (ii), as it is formed in minute amounts, migrates rapidly through the corpuscular wall to be dealt with suitably in the red cell. This is the Hamburger phenomenon, or chloride shift.

Please do not misunderstand the purpose of this paper. It is not intended to be a plea for widespread use of hydrochloric acid as a panacea for all anesthetic faults. It is merely a reference to experimental work already undertaken to determine its value in medicine. You may be assured that I am wholly indifferent as to whether this or some other drug shall ultimately prove to be the answer. We have seen too many failures in our own animal experimentation to believe that the problem is even near complete solution, but the bright spots have urged us to pursue the search.

We believe that ultimately a remedy will be developed that will bring palinesthesia into universal practice. To date, all is experimental.

In our original article,[1] one paragraph has evidently been generally overlooked by all critics of the paper. This paragraph I wish to repeat here: "What may be of greater importance to the public, the anesthetists and surgeons than prompt awakening of patients out of the anesthetic state is that any patient endangered by reason of anesthesia is immediately removed from that danger by the palinaesthesia discovery when it is perfected."

I have made no claim to the effect that palinesthesia has, as yet, been perfected, and we are by no means discouraged by adverse criticism: that was expected and is welcome. Recalling other forward steps in medicine, one is reminded that Pasteur's first public demonstration of smallpox vaccine resulted in the death of fourteen cattle; that Horace G. Wells experienced failure in attempting to demonstrate in Boston that nitrous oxide is an anesthetic agent, and that Morris Richardson of Boston encountered strong resistance in his early effort to persuade the medical profession that appendicitis is an etiologic factor in peritonitis; while, in contrast, most of us can remember the early assurances that "606" (arsphenamine) would banish syphilis from the earth.

It has been suggested by a few that the intravenous injection of even moderate doses of hydrochloric acid diluted to twentieth normal might be distinctly harmful to some individuals, and this may be true unless disproved by further experimental evidence.

It is also true that serum therapy is plagued by its records of fatal anaphylaxis; that arsphenamine has its lethal Herxheimer reaction; that insulin is given not wholly without danger to some, and that all forms of anesthesia, including procaine, have their own unpleasant statistics. But who among you is ready to condemn them entirely, knowing that the lives of many have been made happier because of their use?

Our chief interest is that the principle involved in accomplishing palinesthesia shall be sought for and ultimately perfected for the great benefit that it will bring to that portion of the public who must at some time submit to anesthesia.

DISCUSSION

H. A. Heidbrink, Minneapolis, Minn.: A great deal of interest naturally resulted from the early rumors that a treatment had been discovered with which anesthesia processes might be reversed and anesthetic effects be more quickly terminated. Such treatment, if effective, would doubtless result in the saving of many lives. It is, therefore, somewhat disappointing that Dr. MacGilvra is obliged to abandon the original title to his paper, which implied that these results already had, in a measure at least, been accomplished. Doubtless, Dr. MacGilvra, who was one of the earliest experimenters, is most disappointed because further experimentation indicated that the maximum results hoped for had not yet been accomplished. It is heartening that research is being made in so worthy a field. If, as Dr. MacGilvra contends, intravenous injections of hydrochloric acid lower the thresholds of stimulation, stimulate respiration and elevate blood pressure, it appears that some progress has been made. Some other research workers are not in agreement with Dr. MacGilvra's findings concerning the effects of hydrochloric acid injections following anesthesia in animals on which experiments were made. Shambaugh and Boggs summarize their latest findings as follows[4]: "Observations on guinea-pigs, rabbits, dogs, monkeys and baboons indicate that the intravenous injection of dilute hydrochloric acid is without effect as a means of shortening the recovery period from ether, pentobarbital sodium and tribromethanol narcosis."

Dr. Lundy: I think that Dr. MacGilvra is wrong in his theories and there are a great many others who think so. H. E. Essex, of the Experimental Institute, Mayo Clinic, thinks that, from the results of these experiments, it is quite evident hydrochloric acid is not effective in amytal anesthesia. That is the physiologist's point of view. A physiologic chemist went over the material that has been presented today, and was presented in Indiana and in Boston, with some additions as time goes on,

4. Shambaugh, Philip, and Boggs, Robert: Effect of Intravenous Hydrochloric Acid in Narcosis, J.A.M.A., 102:1912 (April 21) 1934.

and he could see nothing in the interpretation of the data that were presented that would verify the claims that were made. For example, he felt that the p_H of the variation in the blood from the injection of hydrochloric acid in the quantities that were used was not greater than would be found in the normal individuals, and that the amount injected was so small that in the total volume of blood it could not produce the effect. It could not free enough carbon dioxide to produce the effect claimed. Not that there will not be some increase in respiration from a variation in the normal changes in carbon dioxide in the blood stream, but they would not be sufficient to wake the patient or the animal up. From a clinical point of view, I think I could take the same slides that Dr. MacGilvra has presented, and give the same results and show you much more convincingly, to me at least, that the other drugs that were used, for instance, the caffeine, had more to do with sleeplessness than the hydrochloric acid did, and that the injection of epinephrine subcutaneously in the presence of low blood pressure does not raise the blood pressure immediately. In spinal anesthesia, when an extreme fall in blood pressure has been produced, there is no point in injecting epinephrine or anything else subcutaneously. If the effectiveness of the circulatory apparatus is so decreased, it cannot take it up. If the effect cannot be obtained intravenously, it can be had intraspinally, and with much better effect. But if an effect can be obtained intravenously as a rule, intraspinal injection is still more effective. It would be interesting to see whether hydrochloric acid would do the same as ephedrine or epinephrine. It would be more effective. In any animal that is profoundly anesthetized, with fall in blood pressure, and decrease in total volume, any method of increasing depth of respiration and raising the blood pressure will give evidence of a decrease in anesthesia; that is, the patient or animal will temporarily appear to be more lightly anesthetized than before. In the clinical cases, there were measures instituted which should have of themselves produced the same result as was seen here with the hydrochloric acid. I want to show a case of nitrous oxide anesthesia with the blood pressure from the femoral artery, and a normal level from here to the base line (indicating). At this point, an extreme anoxemia is instituted, with the chest torn open and the lungs collapsed so there was no possibility of autoventilation. We see this picture of the blood pressure dropping and going back up again, with many beats of the heart. It goes through one phase and then settles down to a strong pulse pressure with a gradually decreasing blood pressure, until there is not only respiratory collapse, but also stoppage of the heart, which stops by inhibition, and then, with artificial respiration, if the animal can be resuscitated at all, a tremendous effect is evident. In this particular animal, the experiment was repeated, and the animal brought back until blood pressure and pulse rate were normal. An anoxemia is instituted and the heart tends to stop by inhibition. It starts with the final effort to get oxygen, until the blood pressure falls and the heart stops. That is from pure anoxemia and is the typical anoxemia curve. The heart may act in that way in the presence of anoxemia, and I am not sure that this is not what we see in some cases of avertin anesthesia wherein the anoxemia is not recognized because the patient does not go through an acute stage of cyanosis. But the administration of oxygen will bring the patient back to consciousness. Intravenous administration of epinephrine will do the same thing. From Cornell University came sodium rhodanate, and it was supposed to do the same thing, but it does not. We have not been any more successful in the administration of hydrochloric acid than we have been with sodium rhodanate. Nobody would rather have this work prove a success than I, but as far as I can determine, it is not.

Machle-'42
Bib No. 264

J. Ind. Hyg. Tox. 24:222-225. 1942.

THE EFFECT OF THE INHALATION OF HYDROGEN CHLORIDE*

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TOXICOLOGICAL studies on hydrogen fluoride (1) indicated that this gas has two effects, one a primary irritation presumably due to the acidity of the solution formed when it is dissolved in the fluid of the respiratory tract, and a secondary toxic effect caused by accumulation of the fluoride component in the body. It was assumed that if the irritative action of hydrogen fluoride were due entirely to its potentially acid nature, this effect would be comparable to that exerted by hydrogen chloride, since the latter compound does not contain the fluoride ion. Comparable data on the toxicology of hydrogen chloride were not available, consequently it became necessary to investigate the effect of this compound on animals under conditions similar to those used in our studies on hydrogen fluoride, if we wished to compare the two compounds and thus be in a position to differentiate between the two effects of hydrogen fluoride.

EXPERIMENTAL

The same exposure room and sampling apparatus were employed as in the previous experiments (1) but since hydrogen chloride gas was not available in cylinders, it had to be generated by mixing commercial C.P. hydrochloric acid with 10–15 times its volume of concentrated sulfuric acid. A large copper tank (Figure 1) was placed near a wall through which two glass tubes were run, one extending to the bottom of the tank, the other to within 1–2 inches of the surface of the sulfuric acid in the tank. After a vigorous current of air had been started through the sulfuric acid, the hydrochloric acid was run into the tank through the second tube. Amounts of the two acids needed were calculated theoretically, but when analyses showed that all the hydrogen chloride was not liberated from the mixture, an additional amount of hydrochloric acid was added, and after a few trials the desired concentration was easily obtained by empirical calculations. Because of the tendency to dewing on the walls of the chamber, it was impossible to prevent a rapid fall in concentration when the relative humidity in the exposure chamber was above 20–25%; therefore, all the experiments were run during the winter months. Even under these conditions there was a gradual decrease in the gas concentration in the chamber, so that it was necessary to add small amounts of hydrochloric acid to the generating mixture from time to time, but frequent analyses of the mixture enabled us to maintain a fairly constant concentration.

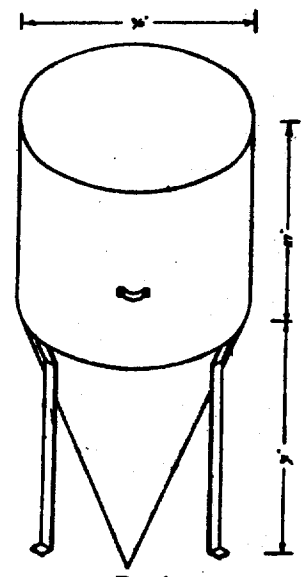

FIG. 1

The apparatus and procedure employed for analyses of the gas mixture were the same as before, except for the substitution of hydrogen chloride solutions for nitric acid solutions, of methyl red for phenol red, and titration at room temperature instead of at 100°. Argentometric titrations were tried in a few instances, but were discontinued after it became evident that they lacked the sensitivity of the acidimetric procedure. Concentrations of hydrogen chloride in the air as given in the results which follow represent averages calculated from 4–160 determinations, depending upon the duration of the experiment.

* Received for publication May 29, 1942. The authors state that 1 mg/l of HCl at room temperature and normal pressure equals 679 p.p.m.

In each of the 37 experiments carried out, three bbits and three guinea pigs were used. The bbits were purchased from local breeders as re some of the guinea pigs, although most of the tter came from the laboratory colony. In the 0-hour experiment, an adult female monkey was so used. The animals were weighed just before e and daily thereafter until they died or had ained their original weight. Animals that vived for two months or longer were sacrificed. e control group consisted of seven rabbits, guinea pigs and one monkey, animals which d been obtained from the same sources and at e same time as the experimental animals.

Discussion

The concentration of the exposure mixtures nged from 0.05–20.5 mg. per liter and exposure

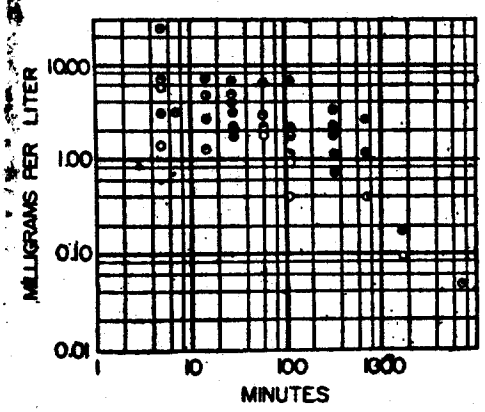

Fig. 2

periods were 5 minutes, 15 minutes, 1 hour, 2 hours, 6 hours, 12 hours, 30 hours, and 120 hours. Exposures for each time period were made with from 1 to 6 different concentrations. The exposures of 720, 1800, and 7200 minutes represent 6-hour exposures carried out on consecutive days for 2 and 5 days respectively, and in the last instance, 5 times weekly for 4 weeks. The results are shown graphically in Figure 2. The diagonal lines mark off three zones: the top zone in which all or nearly all animals died; the lowest zone with no deaths, or rarely a single fatality; and the middle zone with deaths ranging from 1 to 5 in each group. It will be noted that a concentration of 6.5 mg. per liter resulted in a 100% fatality in 30 minutes and that 1 mg. per liter gave the same result if the exposure was of long duration, i.e., from 2–6 hours. The highest concentration that failed to cause any deaths was 5.5 mg. per liter, but the exposure time was only 5 minutes. The longest exposure with no resultant deaths was to a concentration of 0.1 mg. per liter for 1800 minutes, i.e., 5 daily 6-hour exposures. The longest experiment carried out with hydrogen chloride was an exposure to 0.05 mg. per liter for 120 hours (6 hours a day, 5 days a week for 4 weeks). One guinea pig died during the final exposure, but necropsy showed an extensive hemorrhage from the liver probably caused by careless handling. The other animals used for this experiment, including a monkey, showed no evidences of toxic damage when killed several months later. The upper limit of safety in atmospheres containing hydrogen chloride must be, therefore, 0.05 mg. per liter (about 30 p.p.m.), though it is possible that even this concentration would be harmful if daily exposures were continued over periods longer than a month.

In Table 1 the deaths have been tabulated according to their probable cause and the survival time. Apparently the guinea pigs were more severely affected than the other animals by the irritant effects of the gas since 22 of the 57 deaths among them were due to acute respiratory damage. On the other hand, although the rabbits were more resistant to the immediate effects, they died later as the result of pulmonary and nasal infections, to which they were more susceptible. Definite gross pathological changes in the liver were noted in 45 of the 57 guinea pigs that died; in the rabbits, liver damage was much less marked but severe hepatic lesions were found in 16 of the 51 that died. It is possible that some of the deaths occurring after seven weeks were not due to the exposure, but it does not seem advisable to attempt to make any eliminations on this basis.

Two rabbits in the control group died, one as a result of an infection of the mandible, the other from severe intestinal coccidiosis. The rest of the controls were healthy, gained weight and showed few pathological changes on necropsy.

The effect of exposure on the growth of the animals was not remarkable. A sharp loss of weight was evident in every instance and in general paralleled the severity of exposure. Except after the most severe exposures, however, it was rapidly regained after about two weeks by the rabbits, and after one month by the guinea pigs. Following the initial period of loss, growth

of surviving animals was as good as that of the controls.

Only 5 animals, all guinea pigs, died during exposure. All concentrations used were to some extent irritating to the eyes and mucous membranes of the animals. Acute distress was noted with the higher concentrations. Although most of the deaths were due to the respiratory effects, some animals of both species apparently died as a result of liver damage and some of the surviving animals showed gross pathological changes in the liver when killed for necropsy.

A comparison of the results of the experiments with hydrogen chloride and with hydrogen fluoride indicates that the two gases are nearly equal in their immediate toxic action but in respect to permanent effects, the latter compound is somewhat more toxic at all concentrations. The organs of animals surviving exposure to hydrogen fluoride showed more frequent and more severe pathological changes than those of animals exposed to hydrogen chloride. The concentration level which appeared safe for long exposure was also much lower in the case of hydrogen fluoride.

Histopathology

Because of its corrosive action the gas primarily affects the respiratory tract. High concentrations produce necrosis of the tracheal, bronchial and alveolar epithelium, accompanied by extensive pulmonary edema, atelectasis and emphysema. The pulmonary blood vessels share in the damage, there being edema and necrosis of the intima and media, with resultant thrombus formation and frequent pulmonary infarcts. Acute vascular engorgement with capillary venous stasis is frequent, as are hemorrhagic extravasations into the interstitial tissue and pulmonary alveoli.

Severe acute inflammatory reactions were observed in the respiratory tracts of animals which after exposures to high concentrations survived for a few hours or days. These reactions included exudative bronchial inflammation, scattered and confluent lobular pneumonia and frequent large and small bronchopulmonary abscesses. These changes were also frequently encountered in rabbits which lived 5 to 30 days after exposure, but were less frequent in guinea pigs.

TABLE 1

Probable Cause of Death and Survival Time in Days Following Exposure to Hydrogen Chloride

	Days after exposure																					
	0 to 1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 to 22	22 to 29	29 to 36	36 to 43	43 to 50	50 and over	Total
Rabbits																						
Hepatic damage			1	7		1							1	1				1				12
Respiratory infections		1	2	1	1			1	1	3	2	1				3	1			1	4	22
Acute toxic changes in liver and lung	2			2																		4
Nasal infections													1			2	1		1			5
Unclassified																1	1				6	8
Total	2	1	3	10	1	1	0	1	1	3	2	1	2	1	0	6	3	1	1	1	10	51
Guinea Pigs																						
Hepatic damage			5	1	3		1	3	1		1						1	1	1		5	23
Respiratory infections			1	1						1							1				6	10
Acute toxic changes in liver and lung	10	7	1	1	1	1	1															22
Unclassified																					2	2
Total	10	7	7	3	4	1	2	3	1	1	1	0	0	0	0	0	2	1	1	0	13	57

surviving for similar periods. The organization of thrombi and the exudative inflammatory reactions in the vessel walls and perivascular tissue appeared more advanced in the guinea pigs than in rabbits at this period. In several instances, there was extensive proliferation of the capillaries in areas where parenchymal damage had been extreme. Emphysema, atelectasis, chronic purulent bronchitis and peribronchial lymphoid hyperplasia were also frequent in this group. In instances in which exudative bronchial inflammation was not marked, the mucosa was proliferating and often markedly hyperplastic, obliterating the lumina of the medium and smaller branches and extending in neoplastic fashion into the peribronchial tissue.

Similar progressive lesions were encountered in guinea pigs which died or were sacrificed between 1 and 18 months following these exposures. In rabbits of this group lobular pneumonia and multiple bronchoalveolar abscesses were frequent.

Long exposures to low concentrations (less than 1.0 mg. per liter) failed to produce severe lesions in guinea pigs, the changes being limited to mild inflammatory reactions in the bronchi, with some peribronchial fibrosis, and lymph node hyperplasia. In rabbits, however, lobular pneumonia and pulmonary abscesses were common following such exposures.

The occurrence of vascular damage following exposures to high concentrations was suggested by the presence of embolic and thrombotic lesions with infarctions in the heart, liver, kidney, and spleen. After exposures to lower concentrations, there were noted degenerative and necrotic lesions in the intima and media with subsequent medial sclerosis and intimal proliferation. Exudative inflammatory reactions were common in and about the walls of both arteries and veins.

The livers of animals exposed for sufficient periods to high concentrations showed widespread parenchymal edema, congestion, necrosis and hemorrhage. Fatty metamorphosis, common in both species, was more prominent in guinea pigs. In animals which survived such exposures for months, there was observed cirrhotic sclerosis, in some instances associated with slight regeneration of the marginal cells of the lobules, but toxic degenerative changes of varying degree were regularly present in most of the parenchymal cells.

In the kidneys of both rabbits and guinea pigs which survived for several months after exposures to high concentrations, there were focal scarring, chronic cellular infiltration of the interstitial tissue, tubular degeneration and atrophy, hyalin thickening of the glomerular tufts and often complete glomerular sclerosis. After exposures to lower concentrations degeneration and necrosis of the convoluted tubules was often associated with hyalin thickening of the glomerular tufts and with endothelial and epithelial proliferation of the tufts and capsules.

In the heart, there occurred toxic myocardial degeneration and hyalin necrosis, with fibrous replacement and frequent focal and diffuse chronic cellular infiltrations of the muscle bundles and interstitial tissue. There were occasional focal vascular lesions and massive myocardial infarctions.

Summary

A study of the effects of exposure of rabbits and guinea pigs to hydrogen chloride in concentrations ranging from 0.05 to 20.5 mg. per liter for periods ranging from 5 to 7200 minutes has demonstrated that hydrogen chloride has nearly the same immediate toxic action as hydrogen fluoride, but produces less serious residual effects than this compound.

Exposure to hydrogen chloride in a concentration of 6.4 mg. per liter for 30 minutes killed all the animals and the same result followed exposure to 1 mg. per liter for 6 hours.

Three rabbits, 3 guinea pigs, and 1 monkey exposed to 0.05 mg. of hydrogen chloride per liter of air, 6 hours a day, 5 days a week for 4 weeks, showed no immediate toxic effects and no pathological changes which could be attributed to the exposure.

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Matzner-'37
Bib No. 274

Am. J. Digestive Diseases Nutrition 4:180-184. 1937.

Pepsin Versus Hydrochloric Acid in the Experimental Production of Gastric Ulcer*

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THE etiological factors in the production of gastric and duodenal ulcer in man have not been established (1, 2, 3). Peptic ulcers simulating those occuring in man, were first experimentally produced in dogs by Exalto (4) and Mann and Williamson (5). These investigations have been thoroughly reviewed by Graves (6). The effects of acid gastric chyme have been particularly stressed by most authors.

Buchner, Siebert and Malloy (7) have reported that subcutaneous injections of histamine produced ulcers in the prostomach of the rat. These authors as well as Burkle de la Camp (8) contended that the resultant increase in hydrochloric acid and possibly pepsin was responsible for these ulcers.

Both clinically and in animal experimentation the acid portion of the gastric chyme has been regarded as of greater importance than its pepsin content (9). Recent clinical observations of Vanzant, Osterberg, Alvarez and Rivers (10) and confirmed by Mullins and Flood (11), seem to indicate that peptic ulcer in man is frequently accompanied by an increase of pepsin.

It occurred to us therefore that a more direct approach to this problem could be made by controlled feeding experiments on rats, employing solutions of pepsin with hydrochloric acid and hydrochloric acid alone (12).

EXPERIMENTAL

Rats of the original Wistar strain, averaging in age 4 months were fed the Steenbock-Bills diet (13). Fresh lettuce or sliced carrots were added on feeding days. Fluids were supplied by means of stoppered flasks, with glass tube outlets attached to the cages. The rats were then able to partake of these solutions *ad lib.* At first we used a 20% solution of pepsin in 0.3% HCl. We have subsequently found that a concentration of pepsin as low as 1% is equally as effective.

These rats were divided into *two main groups:* a fasting

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This work was aided by a grant from Mr. E. L. Cowen.
Submitted May 19, 1936.

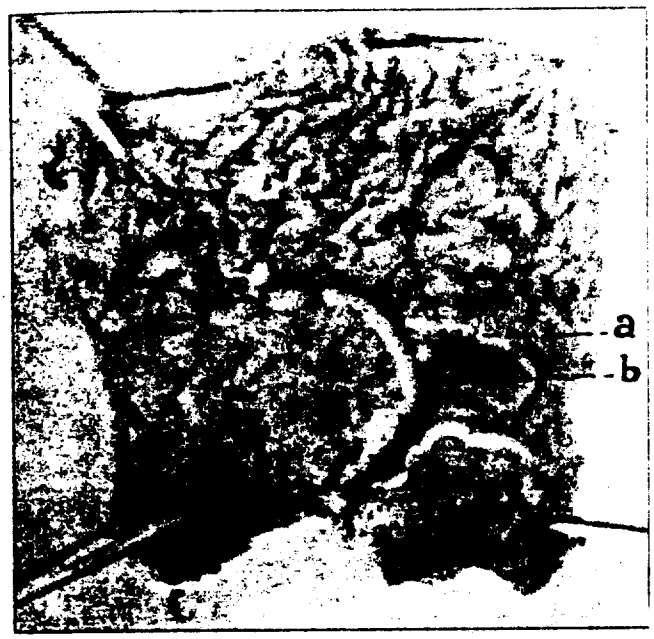

Fig. 1. Photograph of a portion of the pro-stomach of a rat showing a typical ulcer-like, gross lesion of the mucosa. Note the raised margins (a) and central depressed area (b) of the lesion.

and a non-fasting. In the *fasting group*, the rats were fasted for two days and were fed on the third, in recurrent cycles. The *non-fasting group* had continuous access to food. The experimental periods varied from 12 to 21 days, averaging in general about 16 days. The results in the fasting group are summarized in Table I.

RESULTS

In the fasting *Sub-group 1a*, which had continuous access to a 20% (by weight) solution of granular pepsin (1:10,000 Parke, Davis and Co.) in 0.3% hydrochloric acid we found that 96% developed one or more ulcer-like gastric lesions.

On the other hand, in a control *Sub-group 1b* that received only 0.3% HCl under similar conditions, only 16% developed these gastric lesions. In order to establish that the greater incidence in the pepsin hydrochloric acid *Group 1a* was actually due to the activity of the pepsin and not to any foreign substance it might contain, a group of animals received a mixture

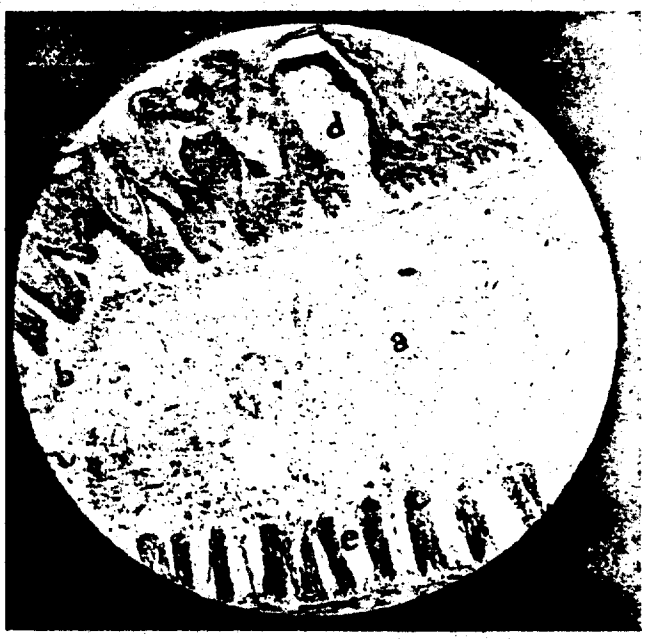

Fig. 3. Photomicrograph of a section of *a typical lesion* described in the gross. Note the marked edema of the submucosa (a) detaching the muscularis mucosa (b) from the underlying muscularis (c). Note also the extent of the infiltrate into the papillary bodies (d) and separation of the bundles of the circular layer of muscle (e).

Fig. 2. Photomicrograph of a section of pro-stomach of submucosa (a) detaching the muscularis mucosae (b) from *a normal rat*. Note the intact submucosa (b) and its close proximity to the overlying mucosa (a) as well as to its underlying muscularis (c). The layers of stratified squamous epithelium are normal in every respect. The superficial cells (d) present only slight desquamation and the basal cell layer (e) is entirely intact.

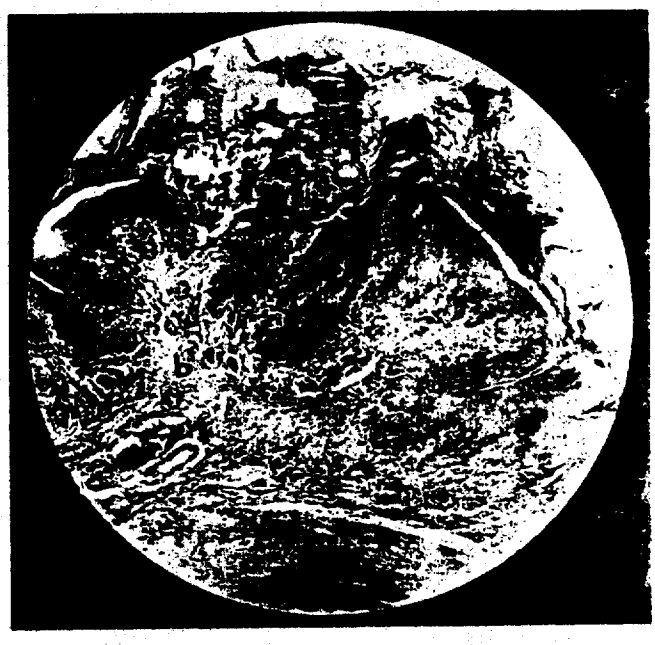

Fig. 4. Photomicrograph of another typical lesion showing marked desquamation of the lining epithelium. Note the point of complete denudation of the lining (a, with arrow) and the congestion and infiltration accompanying the edema in the submucosa (b).

Fig. 5. Photomicrograph of another typical lesion showing the disturbance of the basal layer of epithelium by the advancing infiltrate from the submucosa through the muscularis mucosae (a, with arrow). Note also the moderate degree of desquamation of lining epithelium (b) as well as congestion, edema and infiltration in the submucosa (c).

of inactivated pepsin (heat digestion) and hydrochloric acid. In the latter group we observed lesions in only 20% of the rats. This incidence almost corresponded to that of *Group 1b* that had received hydrochloric acid alone. This appears to establish that the far greater incidence in the pepsin hydrochloric acid group was definitely due to the activity of the pepsin.

That fasting was not the sole cause of these lesions appears to be established by the fact that in *Group 1d* 15 rats with similar fasting periods and continuous access to water alone, no gastric lesions were observed.

The results in the *non-fasting Group 2*, which had continuous access to food are summarized in Table II.

In the *Sub-group 2a*, which had continuous access to a solution of pepsin and hydrochloric acid, we found only 20% developed gastric lesions as compared to 96% incidence in a similar *group 1a* subjected to fasting periods.

Furthermore, no lesions were observed in the *Sub-group 2b* that had continuous access to HCl alone, as compared with an incidence of approximately 16% in rats under similar conditions in the fasting *Group 1b*. Although the absolute incidence of gastric lesions in the non-fasting 2a and 2b is not as great as in the fasting group, the relative incidence is similarly apparent.

To further establish that no foreign substance was present in the pepsin preparation, 20% dry pepsin (by weight) was incorporated in the diet. No gross lesions were observed in any of these rats, 2c. In 60 additional control rats, 2d, on various complete stock diets, no gross lesions were observed at autopsy.

DESCRIPTIONS OF LESIONS

Before describing the observed lesions, the normal anatomical findings in the rats' stomach may be briefly reviewed. The rumen or prostomach is sharply divided from the glanular portion by a smooth, elevated linear ridge, which passes to either side of the cardiac orifice. Histologically, (Fig. 2), the rumen, including the limiting ridge, is lined with squamous epithelium, covered by a thin keratin layer. The epithelium rests almost directly upon the well developed muscularis mucosae, from which it is separated by delicate connective tissue. The muscularis mucosae is separated from the muscularis by areolar connective tissue of the submucosa.

Gross Description: The observed lesions (Fig. 1) were frequently situated in proximity to the limiting ridge and along the lesser curvature. However, other portions of the rumen were often similarly involved. These lesions, which varied in number, were usually discrete, though at times they appeared confluent. Their margins were raised and the base of the umbilicated center appeared brown or red. They measured approximately 0.5 to 5 mm. in greater diameter.

TABLE I

Group 1. Fasting group

Sub-Groups	No. of Rats	B. S. D. = Bills Stock Diet	Mean initial body weight	Mean final body weight	Duration of experiment	Autopsy findings	No. of lesions	No. of rats without lesions	Percentage of rats with lesions
			gm.	gm.	days				
a	30	B. S. D. + .3% HCl + 20% Pepsin. Fasting periods 48 hrs.	190	183	12 to 21	Multiple lesions in 29 rats.	1 to 8	1	96
b	18	B. S. D. + .3% HCl. Fasting 48 hrs.	275	202	21	2 rats—3 lesions. 1 rat—1 lesion.		15	16
c	10	B. S. D. + .3% HCl + 20% inactivated Pepsin. Fasting 48 hrs.			12 to 21	Multiple lesions in 2 rats.		8	20
d	15	B. S. D. + water. Fasting periods of 48 hrs.			28			15	0

TABLE II

Group 2. Non-fasting group

Sub-Groups	No. of Rats	B. S. D. = Bills Stock Diet	Mean initial body weight	Mean final body weight	Duration of experiment	Autopsy findings	No. of lesions	No. of rats without lesions	Percentage of rats with lesions
			gm.	gm.	days				%
a	10	B. S. D. + .3% HCl. 20% Pepsin. No fasting.	165	152	18 to 21	1 rat—5 lesions. 1 rat—1 lesion.		8	20
b	10	B. S. D. + .3% HCl. No fasting.	261	237	21	Negative.	0	10	0
c	8	B. S. D. + 20% Pepsin (dry). No fasting.			28	Negative.		8	0
d	60	Additional controls on various stock diets.			21	Negative.	0	0	0

Microscopic Description: The most constant finding consists of focal areas of submucosal edema, which detach the muscularis mucosae from its underlying coat and which in areas also involve adjacent mucosal as well as muscular layers. In the latter coat, the edema produces separation of the bundles of the inner circular layer of the muscle while in the former (mucosal layer) the edema of the papillary bodies produces wide separation of the epidermal pegs. In most of the areas a pronounced cellular infiltrate and increased vascularity accompany the edema. The exudate consists of extravasated red cells and varying numbers of polynuclear, round and plasma cells (Fig. 3).

The extension of the above described infiltrate of the submucosa into the basal layer of the epidermis, distorting the normal architecture of the latter, especially, in the latter region of the stratum germinativum, is of especial interest.

Other less extensively involved areas, present in addition to the edema, congestion and increased number of blood cells. The above changes are most marked at sites corresponding to the elevated margin of the lesions described in the gross.

A less constant finding consists of desquamation of the lining epithelium in the center of some of these lesions. In areas, the process is advanced to a degree deserving the term "erosion," since the mucosa at these sites is almost completely denuded. At times the desquamation is observed to the degree of complete denudation of the lining membrane.

DISCUSSION

These experiments would seem to prove that pepsin rather than hydrochloric acid is the important agent in the experimental production of gastric ulcer in the rat. While our work was in progress, Howes, Flood and Mullins (14) reported that operatively produced gastric defects in cats showed a greater delay in healing in those receiving pepsin with acid, as compared to those receiving acid alone.

The selective localization of these lesions in the rats' prostomach is of extreme interest. This region is lined by squamous epithelium (a site unusual for peptic ulcer in man). Ulcers only occur occasionally in the glandular portion both in our experiments and in those reported with histamine injections (7, 8). Why histamine (15, 16) and a pepsin containing acid extract of pigs' stomach (17) produced gastric lesions in cats and dogs (lined by glandular epithelium) and why with similar agents in the experimental rat, the prostomach (lined by squamous epithelium) was the seat of election for these lesions, remains unanswered at present.

The dependence of these lesions upon alternating fasting periods, as evidenced by a 96% incidence in the pepsin-HCl fasting group, compared to only 20% in the non-fasting group is likewise very significant. In the light of possible amino acid deficiency, originally suggested by Weiss and Aron (18) experiments are in progress with the simultaneous administration of histidine monohydrochloride and other essential amino acids. The results will be published.

This rapid and simple method of ulcer production in a readily obtainable laboratory animal should afford opportunity for the further study of the role of pepsin in the activation and tendency toward chronicity of peptic ulcer in man.

CONCLUSIONS

1. Pepsin in an acid medium is far more effective than is hydrochloric acid alone in the experimental production of ulcer-like gastric lesions in the albino rat.
2. Fasting appears to be a very important factor.
3. This rapid method of producing ulcers is suggested for the study of the prevention and the treatment of peptic ulcer.

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Bib No. 277

Am. Jour. Physiol. 64:497-507. 1929.

STUDIES ON THE EMPTYING OF THE STOMACH[1]

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Received for publication May 2, 1929

A half-century of experimentation has not solved the problem of the emptying of the stomach. Many factors which might influence the process, as acid, alkali, fluidity, osmotic pressure, and temperature, have been investigated, but in any method of investigation the question always presents itself of how nearly the conditions of the experiment approach those of normal physiologic activity.

My purpose in undertaking this study was to apply to the investigation of the problem a method of fractional gastric analysis which I had devised for the normal dog. The correlation of roentgen studies with the method of fractional analysis enabled me to observe simultaneously the motor and chemical changes which were developing in the organ. The animals were normal healthy kennel dogs, and extraneous factors, such as anesthesia or operation, which might influence the results were not introduced. The quick passage and withdrawal of the stomach tube used in the fractional studies were accomplished with very little apparent psychic effect.

Two series of studies were carried out. In the first series roentgen-ray examinations of the stomach were made to determine the rate of emptying and the type of motor activity at distinct points in the course of digestion. In the second series, the influence of certain factors associated with the process of emptying, namely, free hydrochloric acid, fluidity, and products of digestion was investigated to determine whether any one of them might be the essential factor which controlled the emptying of the stomach.

METHOD OF STUDY. A brief review of the method of fractional gastric analysis is necessary for understanding the sequence of the studies. A meal of meat and water (80 grams of ground horse meat and 250 cc. of water) was fed to a dog which had fasted for eighteen hours. Aspirations were made at regular intervals beginning half an hour after the meal had been eaten. Chemical studies of the aspirated specimens revealed two distinct phases to the digestive cycle. In the two or three specimens aspirated at

[1] Abridgment of thesis submitted to the Faculty of the Graduate School of the University of Minnesota in partial fulfilment of the requirements for the degree of Ph D. in Surgery, 1929.

half-hour intervals first there was a gradual rise in the values of the total acids and neutral chlorides. They rose from an early low level to a high level late in the phase. There was absence of free hydrochloric acid or else it began to appear at the end of the phase. These rising curves were associated with gradual disintegration of the meat to a completely fluid state. As the free acid secreted during this phase was combined with the protein, this was called the "acid-combining phase." During this period the aspirations were made at intervals of half an hour, and there were usually from two to four of them.

Shortly after the total acid curve of the first phase reached a maximal point, the stomach usually had emptied itself completely of its fluid

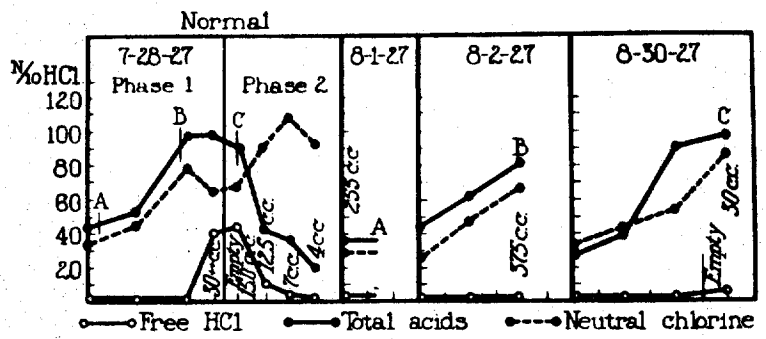

Fig. 1. Normal curve in response to a test-meal of meat. Roentgenograms were made at distinctive points, *A*, *B* and *C*. Chemical analyses of the specimens aspirated at the points at which roentgenograms were made, August 1, 2 and 30, are shown.

chyme and the second phase followed. During this phase only small quantities of clear juice, which contained free hydrochloric acid, could be aspirated. The specimens were aspirated at fifteen-minute intervals during this second phase. Successive aspirations showed decreasing volume. The free acid value rose quickly to a maximum and with the final aspirations fell to the point of neutralization. This second phase is called the "acid-control phase," because the free acid is brought to a point of neutralization by a mechanism discussed in a previous paper (fig. 1).

As these changes occurred in the cycle of digestion, alternations in the motor state of the stomach were suggested by three distinctive conditions encountered by the aspirating tube, within the stomach. Early in the first phase (the first distinctive point), when the digestion of the meat had hardly begun, the tube could not be inserted as far as in the later periods. It was frequently caught in a contracting segment of the stomach, suggesting that the distal end of the stomach was in a state of

vigorous contraction. Later in the first phase (the second distinctive point), when the stomach was distended with fluid chyme, the tube could be advanced farther, and there was not the same evidence of vigorous motor activity in the pyloric antrum. In the second or acid control phase (the third distinctive point), when the stomach contained only small quantities of free acid juice, the organ was collapsed and its walls approximated so that it had to be inflated slightly in order that the small quantities of juice might be aspirated.

Fig. 2

Fig. 3

Fig. 4

Fig. 2. Roentgenogram taken at point *A*, of figure 1, early in the first phase of digestion. Small amount of emptying only a short while after feeding of the meal. Quantity of barium which emptied in a fifteen-minute period is shown.

Fig. 3. Roentgenogram taken at point *B*, of figure 1, late in the first phase of digestion. Large amount of emptying of disintegrated meal in fluid state with high total acids and relaxing pyloric antrum. Quantity of barium which emptied in a fifteen-minute period is shown.

Fig. 4. Roentgenogram taken at point *C*, of figure 1, at beginning of second phase. Almost complete emptying at point where chyme had left stomach; free acid was present, stomach was collapsed and pyloric antrum was relaxed. Quantity of barium which emptied in a fifteen-minute period is shown.

To measure the changing motor activity of the stomach, barium was introduced into the stomach at the three distinctive points in the cycle just described, and a roentgenogram was taken after fifteen minutes had passed. A comparison between the amounts of chyme that emptied into the duodenum during the fifteen-minute interval at these three distinctive points in the cycle, gives a measure of the changing rate of emptying as digestion proceeds. Fluoroscopic observations were made to determine the changes of the motor state of the stomach at these three points in the cycle.

RESULTS IN THE FIRST SERIES OF OBSERVATIONS [illegible]

digestive cycle. (*Fig. 1, A.*) The usual meal of meat and water was administered to a dog which had fasted for eighteen hours. Within fifteen minutes and before any marked digestion had begun, 50 cc. of the meal were aspirated. This was immediately replaced by 50 cc. of a mixture of barium with gum acacia, which kept the volume of the gastric contents unchanged. A roentgenogram was taken after fifteen minutes. Even at this early period some dogs emptied a small quantity of material past the pylorus (fig. 2). Fluoroscopic examination showed that the fundus was distended like a globe, containing most of the meal. It did not exhibit gross peristalsis, only a simple wavering around its circumference. The antrum, from the incisura to the pyloric sphincter, was in a state of constant tonic contraction appearing like a narrow tube with frequent vigorous peristaltic waves traversing it. Frequently two or three waves were visible at once between the incisura and the sphincter. This limited emptying and vigorous motor activity was associated with low total acid and neutral chlorine values, and an absence of free hydrochloric acid (fig. 1, *A*, 8–1–27).

Later in first phase of digestive cycle. (*Fig. 1, B.*) Similar observations were made late in the first phase. On another day, the usual meal of meat was fed to the dog, and three successive aspirations were made at intervals of half an hour. This carried the cycle to a point where the stomach was distended with brown opaque fluid chyme from the disintegrated meat. At the second point late in the first phase, 50 cc. of chyme were aspirated. This was immediately replaced by 50 cc. of barium mixture, again leaving the fluid volume unchanged. A roentgenogram taken after fifteen minutes demonstrated that considerable chyme was passing the pylorus every fifteen minutes (fig. 3) Fluoroscopic study showed that the whole prepyloric segment was considerably relaxed, and that there was less of the vigorous tonic and peristaltic activity which characterizes the early point in digestion. The duodenal cap filled periodically and emptied into the duodenum with very little motor activity in the prepyloric segment. The fundus was diminished in size and still without evidence of significant peristalsis. Chemical analysis of the aspirated specimens showed that there was a marked increase in the total acid and neutral chlorine values, and that the rapid emptying was taking place in the absence of free hydrochloric acid (fig. 1, *B*, 8–2–27).

Second phase of digestive cycle. (*Fig. 1, C.*) At a later date the usual meal of meat and water was administered to the dog. The usual aspirations were made until the point was reached where the stomach was first found to be empty of chyme, and its walls collapsed. The organ was inflated slightly and about 30 cc. of juice (the total content) were aspirated. This aspiration also removed all the air and the walls again collapsed. Chemical analysis of the specimen showed a total acid value of 98 cc. of tenth-normal acid and a free acid value of 8 cc. of tenth-normal acid (fig. 1,

C. S-30-27). One hundred cubic centimeters of barium mixture with a free acid value of 8 was introduced into the stomach. A roentgenogram taken after a fifteen-minute interval showed that in this second phase most of the barium had passed into the intestine in this interval, and the stomach was nearly empty (fig. 4). Apparently at this point there was almost complete relaxation of the antrum which permitted the rapid passage of the barium from the stomach.

Significance of the sphincter. The fluoroscopic studies during early digestion indicated that the whole prepyloric segment is actively involved in the motor activity which regulates the emptying of the chyme. Fractional analysis on dogs whose sphincter had been resected substantiated this fact. In such dogs the meal of meat and water was retained successfully within the stomach so that the usual cycle of chemical changes was observed. However, there were smaller quantities of material retained within the stomach, indicating that the efficiency of the mechanism for the proper retention of the meal had been impaired.

A check by roentgenogram was made on this observation. The usual meal of meat and water was fed to a dog whose sphincter had been resected for about 2.5 cm. After ten minutes 50 cc. of the meal were aspirated and replaced by an equal quantity of barium mixture. A roentgenogram taken fifteen minutes later showed that much larger quantities of chyme passed into the intestine than was noted at this early period of digestion in a dog with the sphincter intact. Although the meal corresponded to that in figure 2, there was more emptying than shown in figure 3. The loss of the sphincter seriously impaired the efficiency of the mechanism which retains the meal within the stomach and yet the prepyloric segment without the sphincter retained a good portion of the meal within the lumen of the stomach.

These observations confirm the idea that emptying of the stomach begins early and continues throughout the whole digestive cycle. Taking three distinctive points in the cycle as isolated points in a gradually progressing phenomenon, roentgenograms indicate that the emptying of the stomach advances at a progressively increasing rate until it is completely empty. This gradual increase in the rate of emptying is accompanied by a progressive disintegration of the meat as measured by the rise in the total acid value and by a gradual reduction of the meal to a fluid state. The faster emptying depends on progressive relaxation of the marked tonic contraction of the pyloric antrum, and reduction of the vigor and frequency of its peristalsis. The sphincter itself seems to fulfill the rôle of the most efficient segment of the antrum. The activity of the whole antrum seems to be as much concerned with the retention of a meal within the stomach until it is digested, as it is with the passage of the products of digestion on into the duodenum.

RESULTS IN THE SECOND SERIES OF OBSERVATIONS. Correlation of these two methods of study on the dog has shown that the progressive rate of emptying of the stomach is accompanied by several constant phenomena: 1, the appearance of free hydrochloric acid closely associated with the terminal period of emptying; 2, progessive disintegration of the meal of meat to an increasingly fluid consistence, until it is reduced finally to a homogeneous viscid fluid state, and 3, changing balance between the raw or partially digested protein of early digestion, and the split products from protein disintegration in later digestion. Further experiments were performed to measure the extent to which any of these factors could determine the motor state of the antrum, and hence the rate of emptying of the stomach.

Acid factor. In a large series of fractional gastric analyses, free acid usually appeared at the beginning of the second phase. Between this point and the preceding aspiration late in the first phase, the stomach had emptied itself of its chyme. The method of study did not give an exact measure of the relationship between the completion of this emptying and the appearance of the acid. However, scattered observations in the course of 275 complete fractional studies (1500 single aspirations) indicated that the appearance of free acid was not an essential factor in bringing about complete emptying of the stomach. A small group of dogs was noted in which considerable free acid was present while the stomach was still distended with a relatively large quantity of fluid chyme. A second group, less frequently noted, was of dogs in which the usual cycle of digestion progressed, the stomach emptied itself, and yet free hydrochloric acid did not appear in the specimen aspirated from the empty stomach early in the second phase. If the presence of free acid controls the emptying process, these two small groups represent a physiologic anomaly.

Careful watch was kept for a dog in the second group which did not show free acid after the stomach had emptied itself in order to make a roentgen-ray observation of the emptying of the stomach in the absence of free acid. In the instances studied the dog was fed the usual meal of meat and water, and the usual aspirations were made during the first phase until the second phase was reached. The first aspiration of the second phase showed that the stomach had successfully emptied itself, yet there was no free acid present in the stomach. Immediately 100 cc. of barium mixture were introduced. A roentgenogram taken fifteen minutes later showed almost complete evacuation of the stomach, as in the average dog, dependent on complete relaxation of the prepyloric segment, with an appearance identical with that shown in figure 4. After the roentgenogram was taken, the barium meal was aspirated. Chemical analysis showed that there was still absence of free acid. So the terminal relaxation was attained and it persisted in these dogs in the absence of free hydrochloric acid.

A second check on the influence of acid toward relaxation of the pyloric

antrum was made by administering a usual meal of meat that had a definite free acid titer. The usual meal of meat and water was acidulated by the addition of free hydrochloric acid, until its free acid value was 16. This is an average value of the juice aspirated after the stomach has emptied itself. It was fed to a dog which had not eaten for thirty-six hours. This longer fast was necessary before the dog would eat the acid meal. A roentgenogram taken after fifteen minutes showed that the free acid could not relax the antrum by overcoming the contractile state established in the presence of the meat as none of the meal passed into the duodenum. The entire meal was aspirated immediately after the roentgenogram was taken, and titration showed that the free acid value was now 9. The presence of this free acid persisted through the fifteen-minute period, yet the vigorous motor activity of the antrum established in response to the stimulus of undigested food persisted despite the presence of the free acid.

The question might be raised whether the action of the free acid on the duodenal side of the pylorus closed the sphincter and prevented the egress of food. Barium mixture with a free acid value of 9 was introduced into a fasting stomach, and a roentgenogram was taken fifteen minutes later. The mixture passed the pylorus readily. This control observation supports the interpretation of the former observation, that the contractile response of the antrum to the stimulus of raw meat predominates over any action that free acid might exert; and the action of the acid in the duodenum did not prevent emptying.

Fluid state. One striking phenomenon in the digestive cycle is the progressive reduction of the meal of meat to its final homogeneous fluid state. Presumably this change in the consistence of the meal might facilitate the passage of the chyme through the pylorus. To measure the influence of the consistence of the meal on emptying, the usual volume of meat was reduced to a fraction of its former volume by dehydration, and it was administered with the usual amount of fluid. This gave a highly fluid meal with a chemically equivalent amount of meat used in all these studies. The usual 85 grams of meat were reduced to a quantity of 30 cc. by drying over a water bath and in an oven. The residue was pulverized and fed to a fasting dog; 255 cc. of barium mixture were introduced with a stomach tube. This method of administration allowed the protein to exert its usual stimulating influence which would have been reduced if suspended diffusely in 255 cc. of barium mixture. A roentgenogram taken after fifteen minutes showed that the meat had again established a state of motor activity in the prepyloric segment which permitted the passage of only a limited quantity of this highly fluid mixture from the stomach corresponding to figure 2.

Products of digestion. Another constant phenomenon observed in using the method of fractional analysis was the disintegration of the raw meat

into intermediary products of digestion as indicated by the rising value of the total acids. To determine whether the products of digestion act in a specific way to stimulate relaxation of the antrum the meat was mixed with the products of late digestion to see if these digestive products could effect passage of the meal from the stomach. The usual meal of meat and water was administered to a dog, and the usual aspirations were made until a late period in the first phase was reached. The whole content of the stomach, 110 cc. of the products of digestion late in the first phase of the cycle, a semifluid mass with a combined acid value of 52, was aspirated. This was used as the fluid vehicle for a test-meal given to another dog. To this partially digested meal, 85 grams of raw meat and sufficient barium mixture to bring the whole volume up to the usual 255 cc. were added; this mixed meal was fed to a fasting dog. A roentgenogram taken after fifteen minutes showed that none of the mixture passed from the stomach. Thus it is seen that the products of late digestion cannot, by any specific stimulus to relaxation, nullify the motor response of the prepyloric segment established in response to the stimulus of raw protein.

COMMENT. In the first series of observations, those made at the three distinctive points in digestion represent isolated periods in a gradually advancing process. They showed that the rate of emptying increased progressively from a slow rate early in the first phase of digestion to a rapid rate late in the first phase. Fluoroscopic studies indicated that the motor activity of the prepyloric segment is probably the chief factor in regulating the rate of emptying. This gradual increase in the rate of emptying is dependent on progressive relaxation of the antrum, and a decrease in the vigor of its peristaltic activity. The motor state of this segment appears wholly independent of that of the fundus. Similarly, Alvarez concluded from his studies that there is "dissociation between the activities of the fundus and the pars pylorica."

Fluoroscopically, the fundus appears as a large motionless ball which contains most of the meal. Peristaltic activity is not marked; simply an irregular wavering is present around its periphery. Alvarez, in studying strips of muscle excised from the fundus, noted that they gave "irregular rounded contractions whose amplitude was small." He continued that the muscle seemed particularly fitted to maintain steady tonic pressure on its contents. In the present studies the fundus became progressively smaller as its contents were diminished, apparently accommodating itself with a type of tonic pressure to the decreasing volume of the meal.

The antrum, on the contrary, was vigorously contracted to a state of "systole," observed by Alvarez (1928), Wheelon (1920, 1922), M'Crea (1924) and others. It was contracted to a small, elongated tube extending from the incisura to the sphincter. Frequent peristaltic waves, often two

to three at a time, traversed this segment. This activity corresponds in a suggestive way to Alvarez's observations with strips of muscles excised from the pyloric antrum. The contractures of these strips when studied by a kymograph made curves of large amplitude, and with sudden rises from the base line. In the present studies the antrum seemed to respond with vigorous tonic and peristaltic activity to the stimulus of the meal, as if it were a more irritable segment than the fundus. Ducceshi observed that the antrum responded more quickly and more energetically to mechanical stimuli than the fundus. It is known that a balloon placed in the pars pylorica will show contractions of that segment, whereas a similar balloon in the fundus will not evoke a similar response. In my studies there appears to be a difference in the irritability of the two segments to the stimulus of undigested meat.

The control of emptying of the stomach cannot reside in the sphincter alone. Resection of the sphincter showed reduction in the efficiency of food retention, but enough food was retained to warrant the conclusion that the whole pars pylorica participates in this control. The sphincter is probably the most efficient segment of the pyloric antrum. From the results of these studies there is no reason to conclude otherwise than that the activity of the sphincter is under the control of the motor activity of the whole phyloric antrum. Wheelon and Thomas, using separate balloons in the antrum and sphincter, demonstrated a coördinated and reciprocal motor activity at the two points. The motor activity in their studies was in response to a constant mechanical stimulus. The present studies have indicated that with a gradual disintegration of the raw protein there is a gradually declining stimulus, so that there is probably a graded and coördinated relaxation of the antrum and sphincter.

In the second part of the studies, different factors observed in the course of digestion were balanced, one against the other, to determine which exerts the greatest influence in altering the motor state of the pars pylorica.

The first factor studied was that of free hydrochloric acid. The most convincing work on the acid control of the pylorus is Cannon's (1911) roentgen studies with various types of foods and his studies with pyloric fistulas, from which he concluded that free acid on the gastric side relaxes the pylorus and on the duodenal side causes its closure. In my studies with fractional gastric analysis progressive emptying during the whole digestive cycle was observed even with the absence of free hydrochloric acid. Further experimental study showed that free acid could not relax the segment in the presence of the stimulus to contraction from raw undigested protein. The usual vigorous motor activity characteristic of early digestion was established despite the presence of free hydrochloric acid, and no part of the meal left the stomach. Furthermore, dogs were ob-

served who completed the digestive cycle and completely emptied the stomach without the appearance of free hydrochloric acid. These results of study on a large series of dogs with the use of fractional test-meals, indicate that the appearance of free hydrochloric acid is probably more an associated phenomenon then a regulatory one. It is dependent, probably, on the persistence of an elevated rate of secretion of free acid, after all the food that can combine with it has passed from the stomach.

It is a widely accepted conception that the more fluid the consistence of a meal, the more rapidly it will empty from the stomach. My studies show, however, that raw protein can establish motor activity in the pyloric antrum which will prevent the rapid egress of an almost wholly fluid meal so that consistence alone does not suffice to control emptying. In the course of normal digestion, however, these two factors do not have the antagonistic relationship of this study. On the contrary, as the fluidity of the meal increases, there is a parallel disintegration of the raw protein to the products of digestion late in the first phase of the cycle. This increased fluidity probably facilitates the passage of the contents of the stomach through the pyloric antrum, which gradually relaxes as the raw protein is disintegrated. In this study there was no evidence to indicate that the products of digestion at this point can act in a specific manner to stimulate relaxation of the pars pylorica.

SUMMARY

The method of study by the combined use of fractional analysis and of roentgenograms has allowed close correlation between the changes in the motor activity of the stomach and the parallel changes in the chemical status of the gastric contents. It has permitted a comparative study of the influence of various factors on the motor state of the stomach so far as these factors might control the mechanism of emptying.

The rate of emptying of the stomach increases progressively from early digestion until the process is complete. It depends on progressive relaxation of the pars pylorica of which the sphincter is the most efficient segment. The mechanism for control does not appear to be a specific stimulus to relaxation of the segment by free hydrochloric acid or the products of digestion. Rather does the activity of the antrum seem to depend on its irritability, and, in these studies, on the stimulating action of raw protein to vigorous tonic and perist[illegible]tion. With the gradual disintegration of the protein to the products of digestion late in the first phase of the digestive cycle, there is apparently a graded reduction in the intensity of the stimulus to the antrum and consequently a progressive relaxation of theantrum with more rapid emptying The parallel change of the meal to a fluid state probably facilitates the emptying of the stomach.

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McClure-'20
Bib No. 279

Arch. Int. Med. 26:410-423. 1920.

ON THE BEHAVIOR OF THE PYLORIC SPHINCTER IN NORMAL MAN *

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Systematic studies of motor phenomena of the normal gastro-intestinal tract have been so largely carried out in the lower animals that but little detailed description of them in man is available. The results obtained from the studies in the lower animals can be applied only in a general way to the physiology of the digestive tract in man. It follows that more intimate knowledge concerning the motor phenomena of the alimentary canal in man would aid in the interpretation of results obtained from experimental studies of various phases of the pathologic physiology of human digestion. For this reason the work, with which the present report deals, was undertaken. It comprises observation on the behavior of the pyloric sphincter of the normal man in relation to various foodstuffs, and also to the presence of acid and alkaline solutions introduced into the stomach and duodenum of persons whose gastro-intestinal tracts were considered normal.

The results obtained from the latter observations bear particularly on the question of the "acid control" of the pyloric sphincter. The theory of the "acid control" of the sphincter was suggested by Pawlow,[1] among others, and was elaborated later by Cannon.[2] In epitome the theory is that the presence of acid in the antrum of the stomach causes relaxation of the pyloric sphincter, while the presence of acid in the duodenum causes the sphincter to contract. The evidences on which this theory are based were, again, obtained from experiments carried out on the lower animals. There are, however, phenomena occurring in the lower animals which it is admitted that the theory of acid control does not explain, and for this reason other controlling factors are assumed to exist. Whether or not acid or some other factor exerts the principal control of the pyloric sphincter in man has not been demonstrated by previous investigators.

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* From the medical clinic and roentgenographic department of the Peter Bent Brigham Hospital.

1. Pawlow: The Work of the Digestive Glands, London, 1902, p. 165.
2. Cannon, W. B.: The Acid Control of the Pylorus, Am. J. Physiol. **20**: 284, 1907.

METHOD OF OBSERVATION

1. The degree of contraction of the pyloric sphincter when foodstuffs partially fill the normal stomach was studied.

The data for this were obtained from fluoroscopic observations of normal human stomachs.

2. The behavior of the sphincter relative to the passage of carbohydrate, protein or fatty foods from the normal stomach into the duodenum was studied.

This was determined by fluoroscopic observations of the stomachs of normal persons after feeding meals composed of thick oat-meal porridge and barium, or 140 gm. of ground lean meat and 40 gm. of barium sulphate baked into a loaf, or 120 gm. of ground fatty bacon, 5 egg yolks and 40 gm. of barium baked into a loaf. The protein and fatty meals were of constant bulk and consistency.

3. Effect of the application of hydrochloric acid and sodium bicarbonate solution to the antral and duodenal ends of the pyloric sphincter on its functioning was studied.

Observations of the functioning of the sphincter were made by means of the fluoroscope.

The duodenal tube was given on an empty stomach and allowed to pass into the antrum in the region of the sphincter, or into the duodenum. Then a meal was fed. This was a meat and barium meal, either rinsed down with 200 c.c. of water or made into a thick mush by grinding in a mortar with water. In certain cases a malted milk, potato starch and barium mixture was also given. After the meal had been ingested fortieth normal, twentieth normal and tenth normal hydrochloric acid and 1 per cent. or 5 per cent. sodium bicarbonate solutions were introduced into the sphincteric region of the antrum or into the various portions of the duodenum, through the duodenal tube.

The subjects used were persons whose physical condition at the time of the observations was considered to be normal. The usual fractional clinical gastric analysis (Ewald test breakfast) showed no abnormalities.

1. The study of the degree of contraction of the normal pyloric sphincter, observed by means of the fluoroscope.

That the pyloric sphincter is closed when fluid food is in the stomach, except during the periods in which it opens to permit the ejection of food into the duodenum, was demonstrated in 1913 by Cole.[3] His observations were so readily and easily confirmed by fluoroscopic studies that they are now universally accepted. In this way the authors have confirmed Cole's observations a large number

3. Cole, L. G.: Physiology of the Pylorus, Pilleus Ventriculi and Duodenum as Observed Roentgenographically, J. A. M. A. 61:762 (Aug. 29) 1913.

of times. Further evidence that the normal quiescent sphincter is in a state of contraction is afforded by the following facts. By the use of considerable pressure on the abdominal wall, barium containing chyme can be forced from the stomach into the duodenum, as observed by means of the fluoroscope. This can be accomplished probably only when the sphincter has opened in relation to the advance of an antral peristaltic wave. When it can be accomplished the filled antrum and first portion of the duodenum are seen to be connected by a narrow isthmus of barium, which represents the sphincter; on cessation of pressure this narrow isthmus disappears.

When a fluid barium meal first enters the antrum of the stomach it frequently passes directly into the duodenum. Careful observation has shown that the barium does not fall through an open sphincter by means of gravity, but that from one to four small peristaltic waves are present in the antral region and that the pyloric sphincter opens in relation to the advance of a peristaltic wave; which latter seemingly ejects the barium into the duodenum.

2. The study of the behavior of the pyloric sphincter, observed by means of the fluoroscope, relative to the passage of carbohydrate, protein and fatty foods from the stomach into the duodenum.

By means of the fluoroscope a fluid carbohydrate meal, consisting of 500 c.c. of malted milk in which 90 gm. of barium sulphate are held in suspension by the aid of potato starch, is seen to start through the normal pyloric sphincter from at once to within a few minutes after it has been ingested. The normal sphincter opens regularly as each antral peristaltic wave approaches and permits the passage of barium containing chyme into the duodenum over a period of about ten seconds. In two normal young men the same phenomena occurred after the ingestion of thick oatmeal porridge mixed with barium. Except in pathologic conditions accompanied by pylorospasm, opening of the pyloric sphincter at irregular intervals, as described by Cannon in cats, was not observed. The regular opening and closing of the pyloric sphincter, except in the presence of pylorospasm, is so well known after the ingestion of fluid carbohydrate meals that no further observations on them were attempted in the present investigation.

Observations on the behavior of the pyloric sphincter after the ingestion of protein food were made in young men, ten normal, one with a quiescent duodenal ulcer, and one woman whose physical condition was normal. Similar observations were made after the ingestion of fatty food in eight normal young men. The duration of each experiment varied from one-half hour to six hours.

Since the results obtained in the various subjects were consistently uniform, only three of the experiments will be given in the following illustrative protocol.

REPORTS OF OBSERVATIONS

PROTOCOL I.—*Observation* 1.—Male, aged 25.

MEAT MEAL

Meat and barium meal with 100 c.c. of water to drink.
5:25 p. m.: Began eating meal.
5:35 p. m.: Finished eating the meal.
Fluoroscopic observations; subject in reclining position.
5:35-5:38 p. m.: Gastric peristalsis active. As each wave approached the pyloric sphincter barium passed into the duodenum over a period of about 10 seconds.
5:45-5:47 p. m.: Ditto.
5:53-5:55 p. m.: Ditto.
6:00-6:05 p. m.: Ditto.

FATTY MEAL

Fatty and barium meal with 100 c.c. of water to drink.
6:07 p. m.: Began eating the meal.
6:14 p. m.: Finished eating the meal. The meal was nauseating to patient since he did not like fat; no nausea present after having eaten the meal.
Fluoroscopic observation; subject in erect posture.
6:14-6:16 p. m.: Gastric peristalsis active. As each wave approached the pyloric sphincter barium passed into the duodenum over a period of about 10 seconds.
6:21-6:24 p. m.: Ditto.
6:29 p. m.: Ditto.

Observation 2.—Male, aged 22.

FATTY MEAL

Bacon, egg and barium meal eaten as a dry loaf and without water to drink.
9:21 a. m.: Began eating the meal.
9:29 a. m.: Finished eating the meal.
Fluoroscopic observations; subject in erect position.
9:29-9:40 a. m.: Gastric peristalsis active. As each wave approached the pyloric sphincter barium passed through into the duodenum.
10:02-10:04 a. m.: Ditto.
10:28 a. m.: Ditto.
11:02 a. m.: Ditto.

Observation 3.—Male, aged 24. Meat and barium meal eaten as a dry meat loaf and without water to drink.
9:47 a. m.: Began eating the meal.
9:57 a. m.: Finished eating the meal.
Fluoroscopic observations; subject in erect position.
9:57 a. m.: Gastric peristalsis active. As each wave approached the pyloric sphincter barium passed through into the duodenum.
10:17-10:23 a. m.: Ditto.
11:09-11:12 a. m.: Ditto.
11:40-11:42 a. m.: Ditto.
12:35-12:37 a. m.: Ditto.

Observations on the various subjects, similar to the ones illustrated in the foregoing protocol, demonstrated that either the protein or the fatty meals began to leave the stomach within from three to ten minutes after the first mouthful of food was ingested; that is, as soon as the subject could ingest the meal and be prepared for fluoroscopic observation. The consistency of the food did not modify its initial leaving-

time. Furthermore, the food passed through the pyloric sphincter into the duodenum as each and every antral peristaltic wave approached that orifice, and during a period of about ten seconds. In the same subject no difference in the amounts, either of the same food or of the different foods, which passed through the sphincter at different times, was observed.

3. Effect of hydrochloric acid and sodium bicarbonate solutions on the opening and closure of the pyloric sphincter, as observed through the fluoroscope.

In observations on two subjects from 10 to 20 c.c. portions of twentieth-normal hydrochloric acid were repeatedly poured into the stomach almost on the antral end of the pyloric sphincter. No effect on the opening or closure of the latter could be observed. In a third subject a loop of the duodenal tube entered the duodenum in such a manner as to pull the metal tip up against the antral end of the sphincter. The pouring of 20 c.c. of this acid on the antral end of the sphincter resulted in almost complete cessation of gastric peristalsis on one occasion and, on another, complete pylorospasm developed without demonstrable disturbance in peristalsis. The introduction of 20 c.c. of 1 per cent. sodium bicarbonate solution was immediately followed by acceleration of gastric peristalsis; i. e., the duration of a wave decreased to thirteen seconds from twenty-two seconds. The action of the pyloric sphincter, as judged by its regularity of opening and the amount of barium which passed through it, was not affected. In an observation on another subject, 5 per cent. sodium bicarbonate solution was poured into the sphincteric region of the antral end of the stomach. The result obtained is outlined in Observation 3 of Protocol II.

The effect of the introduction of acid and alkaline solutions into the duodenum is outlined in Protocol II.

PROTOCOL II.—*Observation* 1.—Male, aged 26.

The patient complained of belching, occasional vomiting, pains in various regions of the abdomen associated with bowel movements, constipation, shooting pains in the chest, back and legs, and insomnia. Diagnostic studies were all negative After an Ewald test breakfast free hydrochloric acid was 15 and total acidity was 25. The patient was observed for two months and no evidence of organic disease was elicited during this period.

1:00 p. m.: Light lunch of toast, cereal and coffee. Duodenal tube swallowed.

4:00 p. m.: Subject was given 500 c.c. of barium, malted milk, potato starch mixture.

Fluoroscopic observations; subject in erect posture.

4:05 p. m.: Metal tip of duodenal tube in third portion of duodenum. It was pulled back into the first portion to a point just beyond the sphincter. Active peristalsis. Barium passed over in normal amount as each peristaltic wave approached the pyloric sphincter; 5 c.c. twentieth normal hydrochloric acid was quickly introduced through the tube as a gastric peristaltic wave

approached the sphincter and before the proper time for the sphincter to open. The sphincter opened and a normal amount of barium passed through into the duodenum.

Second observation twenty-four hours later.

Subject was not given food or drink for fourteen hours prior to introducing the duodenal tube.

8:25 a. m.: Duodenal tube swallowed; 85 c.c. of fasting gastric contents aspirated containing free hydrochloric acid 20.

11:25 a. m.: Meat and barium meal eaten. Metal tip of duodenal tube found in third portion of duodenum. Gastric peristalsis active. Barium passed over in normal amount as each peristaltic wave approached the sphincter. Five c.c. twentieth normal hydrochloric acid was introduced through the tube a short period before the proper time for the sphincter to open and when the duodenum was empty. Gastric peristalsis, the opening of the sphincter and the amount of barium which passed over were unaffected.

11:30 a. m.: Six c.c. twentieth normal hydrochloric acid were again introduced into duodenum a short period before the proper time for the sphincter to open and when the duodenum was empty. The same result occurred as in the preceding observation.

11:35 a. m.: The metal tip was pulled back into the first portion of the duodenum to a point just beyond the opening of the sphincter. Six c.c. twentieth normal hydrochloric acid were quickly introduced a short period prior to the time that the pyloric sphincter should open and when the duodenum was empty. The time of opening of the sphincter, the amount of barium passing through it, and gastric peristalsis were unaffected.

Observation 2.—Male, aged 28.

The subject complained of more or less constant mild headache. Diagnostic studies were negative. Fasting gastric contents: amount, 30 c.c.; free hydrochloric acid, 40; total acidity, 50. The patient had had a lumbar puncture several days prior to the observation. As a result he had a headache when in the erect posture, which disappeared completely on reclining. On the morning of the observation patient was given neither food nor drink.

9:00 a. m.: Duodenal tube swallowed.

10:10 a. m.: Began eating the meat and barium meal, which was ground up with water into a very thick mush.

10:17 a. m.: Finished eating the meat meal.

Fluoroscopic observations; subject in erect posture.

10:17 a. m.: Metal tip of duodenal tube in duodenum. Antral peristaltic waves very shallow.

10:25 a. m.: Antral peristalsis very weak, but a small amount of barium passed into the duodenum as each antral peristaltic wave approached the sphincter. Metal tip pulled back into third portion of duodenum.

10:40 a. m.: Subject nauseated, with moderately severe headache. Gastric peristaltic waves very shallow, and no barium passed through the sphincter. The metal tip was now found in the first portion of the duodenum just beyond the end of the sphincter.

The subject was now placed in reclining position on horizontal fluoroscopic table. His nausea and headache disappeared and about 15 minutes later antral peristalsis was active, and barium passed into the duodenum in easily demonstrable amount as each antral peristaltic wave approached the pyloric sphincter.

Fluoroscopic observations; subject in reclining position.

Metal tip in first portion of duodenum. Ten c.c. fortieth normal hydrochloric acid introduced beginning about five seconds before, and continued up to the time of opening of the sphincter. The sphincter opened normally, and also at the approach of the next two antral peristaltic waves.

Fifteen c.c. fortieth normal hydrochloric acid were now introduced under the same conditions as before. Sphincter opened normally and also at the

approach of the next four antral peristaltic waves. This procedure was now repeated twice with 20 c.c. of twentieth normal hydrochloric acid and with same result as noted before.

Twenty c.c. of 5 per cent. sodium carbonate solution were now introduced the latter part of the time the sphincter was open and up to the time of its complete closure. The sphincter closed normally and reopened normally. This procedure was repeated with 10 c.c. and then 15 c.c. of 5 per cent. sodium bicarbonate solution. The opening and closure of the sphincter were unaffected.

The duodenal tube was now washed with 60 c.c. of water (in 20 c.c. portions). For a few minutes following this, antral peristaltic waves became very shallow.

11:45 a. m.: Antral peristalsis active. Barium passing through sphincter in considerable amount with each antral peristaltic wave. During the periods barium was passing through the sphincter into the duodenum on three successive times the contents of the first portion of the duodenum were aspirated. Eight c.c. were obtained. This material was liquid, containing a small amount of barium and of meat fibers. Its acidity was: free hydrochloric acid, none (Töpfer's reagent); 5 c.c. neutralized 1 c.c. of tenth normal sodium hydrate. Hydrogen ion concentration = 5.60 (determined by potentiometer).

Observation 3.—Male, aged 47.

Subject had a mild type of chronic arthritis. At the time of the following observation the subject was in good health.

7:00 a. m.: A light breakfast was eaten.

1:00 p. m.: Duodenal tube swallowed, followed by a glass of water.

5:35-5:40 p. m.: Subject given one half of meat and barium meal and 450 c.c. of malted milk, potato starch and barium mixture.

5:48 p. m.: Antral peristalsis weak and but small quantities of barium pass into the duodenum as each peristaltic wave approaches the sphincter. Metal tip of duodenal tube in third portion of duodenum.

6:00 p. m.: Metal tip of duodenal tube pulled back into first portion of duodenum. This act resulted in almost complete cessation of gastric peristalsis for several minutes. After peristalsis again became active barium passed into the duodenum as each antral peristaltic wave approached the sphincter. On one occasion 5 c.c. and on three occasions 10 c.c. of fortieth normal hydrochloric acid and on one occasion 12 c.c. of twentieth normal hydrochloric acid (all warmed to 38 C.) were introduced into the first portion of the duodenum through the duodenal tube. Each portion of acid was introduced before and up to the time of opening of the sphincter in relation to the advance of an antral peristaltic wave. The introduction of the acid was not followed by a demonstrable effect on the opening of the pyloric sphincter, the amount of barium passing through it into the duodenum or on gastric peristalsis.

7:00 p. m.: Metal tip of duodenal tube pulled back into stomach and lay at the pyloric orifice of the sphincter. Forty c.c. of 5 per cent. sodium bicarbonate solution were introduced through the duodenal tube. The pyloric sphincter continued to open at the approach of antral peristaltic waves and barium was ejected into the duodenum in normal amount. Then the duodenal tube was washed by injecting 10 c.c. of water through it. Then 10 c.c. of gastric contents were aspirated and a few minutes later 2 c.c. more were withdrawn. Both these specimens were faintly alkaline to phenolphthalein.

Observation 4.—Male, aged 21.

Six years ago temporary jaundice had occurred following an attack of malaria. Beginning two years ago and lasting until one year ago there had been attacks characterized by nausea, vomiting and diarrhea. These attacks occurred at intervals of from two to three months and persisted for from one to seven days. There had been no hematemesis, and no bloody, tarry or clay-colored stools. Six months ago jaundice again recurred. During this time there had been constipation.

Physical examination was negative, except for slight icterus of the sclerae. The laboratory examinations were negative. The urine contained no bile pigments. After an Ewald test breakfast the gastric contents showed free hydrochloric acid 14 and total acidity 70.

At the time of the present observation jaundice of the sclerae had disappeared and the patient was free from symptoms.

On the morning of the observation the subject was given neither food nor drink.

8:00 a. m.: Duodenal tube swallowed, followed by a glass of water.

10:15-10:20 a. m.: Meat and barium meal eaten with 200 c.c. of water to drink.

Fluoroscopic observations; subject in erect posture.

10:30-10:40 a. m.: Metal tip of duodenal tube is in the third portion of the duodenum.

Gastric peristalsis regular and active. Pylorospasm was present and no barium passed through into the duodenum.

10:47-11:00 a. m.: Peristalsis active. A very slight amount of barium passed through the sphincter as each peristaltic wave approached that orifice. Subject placed on right side in the reclining position.

11:02-11:20 a. m.: Subject in the reclining position. Fluoroscopic findings during this period remain the same as in the preceding observation.

11:35 a. m.: Subject in the reclining position usual for fluoroscopic gastric examination. Metal tip of duodenal tube was at the junction of the second and third portions of the duodenum. Five c.c. of twentieth normal hydrochloric acid were slowly introduced beginning at the time of the starting of a peristaltic wave in the antrum and continued until a second similar wave had reached the pylorus. Both waves pushed barium through the sphincter in normal amount. The metal tip of the duodenal tube was now pulled back until it lay in the first portion of the duodenum, just beyond the end of the pyloric sphincter. Following this procedure gastric peristalsis ceased, except for some very shallow, feeble waves, which sent but a very small amount of barium through the sphincter into the duodenum.

After the lapse of a period of thirty minutes peristalsis again became active and barium in normal amount passed through the pyloric sphincter into the duodenum. Then 20 c.c. of twentieth normal hydrochloric acid were introduced slowly and continuously from the beginning of one antral wave until the end of the third. All three waves, as they approached the sphincter, were followed by the passage of a normal amount of barium into the duodenum. The same procedure was immediately repeated with a second 20 c.c. amount, of twentieth normal hydrochloric acid and with the same result as noted before. A few minutes later peristaltic waves became weak and feeble and sent but a very small amount of barium through the sphincter.

After a period of about five minutes peristalsis again became active and barium passed through the sphincter in normal amount. The same procedure was repeated with 20 c.c. tenth normal hydrochloric acid and with the same results as before. After a few minutes peristaltic waves again became shallow and feeble and sent but small amounts of barium through the sphincter.

After about five minutes peristalsis again became active and barium passed through the sphincter in normal amount as each peristaltic wave approached that orifice. The same procedure was repeated with 20 c.c. of 1 per cent. sodium bicarbonate solution. No effect was noted on gastric peristalsis, the time or manner of the opening or closure of the sphincter or on the amount of barium which passed through it into the duodenum.

Observation 5.—Male, aged 35.

The subject had suffered with acute articular rheumatism, from which he had promptly recovered. At the time of the observation he was in normal health.

The subject was given neither food nor drink on the morning of the observation.

9:10 a. m.: Duodenal tube swallowed.

Fasting contents—amount 100 c.c., free hydrochloric acid, 30; total acidity, 60.

11:00 a. m.: Began eating one half of a meat and barium meal ground up with water into a thick mush.

11:07 a. m.: Finished eating half of the meal.

Fluoroscopic observations; subject in erect posture.

11:07 a. m.: Metal tip at first was in the third portion of the duodenum, and then slowly receded to the first portion. The movement of the metal tip was accompanied by pylorospasm which continued for ten minutes after its movement ceased. Antral peristalsis then became active and barium passed from the stomach into the duodenum as each peristaltic wave approached the pyloric sphincter.

Ten c.c. fortieth normal hydrochloric acid were introduced into the first portion of the duodenum before each opening of the sphincter in relation to the advance of two consecutive antral peristaltic waves (the acid was introduced before and up to the time of opening of the sphincter). No effect was observed on the opening or closure of the sphincter immediately following the introduction of the acid or its opening in relation to the advance of the three succeeding waves. Following the latter antral peristaltic waves became shallower and less barium passed through the sphincter. After peristalsis again normal 12 c.c. fortieth normal hydrochloric acid introduced under the same conditions. No effect on the opening of the sphincter in relation to the next four waves was observed. The patient now coughed and the next two waves were very shallow; but the next waves were again normal. Then 20 c.c. fortieth normal hydrochloric acid were introduced, and no effect on the opening of the sphincter in relation to the next six waves was observed.

12:10 p. m.: Duodenal tube washed with 5 c.c. of water and then emptied with air. Chyme flowing into the first portion of the duodenum on two consecutive openings of the sphincter was aspirated, about 8 c.c. were obtained. Subject was now given 300 c.c. of malted milk, starch and barium mixture. Then 20 c.c. of fortieth normal hydrochloric acid were introduced into the duodenum prior and up to the time of opening of the sphincter. No effect on the behavior of the sphincter was observed. Twelve c.c. of twentieth normal hydrochloric acid were now introduced as before, without effect on the opening of the sphincter. Ten c.c. 5 per cent. sodium bicarbonate solution were introduced into the first portion of the duodenum just before and during the opening of the sphincter and continued up until it closed completely. The sphincter closed normally. Twenty c.c. 5 per cent. sodium bicarbonate solution were introduced during the period the sphincter was open and up until it had closed. The sphincter closed normally.

In terms of normal acid the aspirated duodenal contents contained five hundredth normal free hydrochloric acid; the total amount of sodium hydrate neutralized was equivalent to an acidity of twenty-fifth normal, titrated to phenolphthalein.

DISCUSSION

In the observations outlined in the above protocol twenty-two observation were made on the effect of introducing acid solutions into the first, second or third portions of the duodenum. The time of the introduction of the acid in relation to the normal opening time of the pyloric sphincter varied. Acid was introduced: (1) a short period prior to the normal opening time of the pyloric sphincter in relation

to the advance of an antral peristaltic wave; or (2) at the time just mentioned and continued until after the sphincter had opened for a second or third successive time.

In all the observations of the above protocol, the introduction of acid into the duodenum had no demonstrable effect on either the opening or the closure of the pyloric sphincter, on the amount of barium containing chyme ejected into the duodenum, or on gastric peristalsis. In Observations 4 and 5 the introduction of acid into the duodenum had no initial effect, but a late effect was noted. The latter consisted of partial cessation of gastric peristalsis. Moving the duodenal tube produced the same effect in these two observations and also in Observation 3, while in Observation 5 it produced pylorospasm on one occasion. These phenomena were interpreted as the result of abdominal irritation of the mucosa of the sphincter and duodenum due to movements of the tube. On this basis the partial cessation of gastric peristalsis, in Observations 4 and 5, may be explained; movements of the tube may have been produced by gastric and duodenal peristalsis. In this connection possible irritation of the duodenal mucosa incident to the introduction of fluid, its temperature, or its acid character must be considered. Irritation incident to the presence of the tube in the sphincter and duodenum can explain the pylorospasm present on the first observation in Observation 4. In two duodenal observations, on two subjects not outlined in the above protocol, the introduction of 20 c.c. amounts of twentieth normal hydrochloric acid into the first portion of the duodenum resulted in duodenal antiperistalsis associated with either prolonged pylorospasm or almost complete cessation of antral peristalsis. These phenomena were considered as the result of abnormal irritation of the duodenal mucosa resulting from the introduction of the acid. It will be remembered that mention has already been made of the development of pylorospasm and of partial cessation in gastric peristalsis after pouring twentieth normal hydrochloric acid onto the antral end of the pyloric sphincter.

The introduction of 5 per cent. sodium bicarbonate solution into the first portion of the duodenum in Observations 2 and 5, and 1 per cent. solution in Observation 4, produced no effect on the opening or closure of the pyloric sphincter, on the amount of barium passing into the duodenum, or on gastric peristalsis.

SUMMARY

Observations illustrated in Protocol I demonstrate that finely divided foodstuffs, either of carbohydrate, protein or fat, do not differ in the initial time at which they begin to leave the stomach and pass into the duodenum. This initial leaving time was not affected by variations in the consistency (viscosity) of the various foodstuffs. The food

was passing through the sphincter by the time the subjects could ingest the meals and be prepared for fluoroscopic observation; i. e., in from three to twelve minutes.

The barium containing chyme passed through the pyloric sphincter as each and every peristaltic wave approached that orifice. It continued to pour through the sphincter for a period of about ten seconds and then closed completely. The opening of the pylorus at irregular intervals, as described by Cannon[4] in cats, was not observed. The regular opening of the sphincter in man was observed by Cole,[5] but unfortunately his publication gives no experimental details.

In the five duodenal observations outlined in Protocol II the introduction of acid into the duodenum did not prevent the opening of the sphincter, or produce its closure. The amount of free acid present in the first portion of the duodenum is slight; in two subjects it was present in the concentration of eighty-fifth normal and five hundreths normal, and in another (Observation 2) no free hydrochloric acid was present. The acidity as titrated to phenolphthalein is low; expressed in terms of normal acidity, twenty-fifth normal, fiftieth normal and [illegible]ntieth normal were found. These findings confirm those of [illegible]cClendon and Myers.[6] The latter observers determined the hydrogen ion concentration of the duodenal contents. Their findings showed that a low degree of acidity was usually present. From this discussion it is obvious that the amount of acid introduced in these observations [illegible] always much in excess of the amounts of free hydrochloric acid [illegible]nt normally in the duodenum and its titration value varied from [illegible]esser amount to an excess in relation to the total acidity, titratable to phenolphthalein, normally present. Furthermore, as shown in Observation 2 of Protocol II the titration value of the total acidity of the duodenal contents is no index to its hydrogen ion concentration. It may, therefore, be stated that the amount of acid introduced was always of a hydrogen ion concentration much in excess of that normally present in the duodenum. These observations do not support the theory that acid in the duodenum is the principal factor causing contraction of the pyloric sphincter.

In Observations 2 and 5 chyme was collected as it entered the first portion of the duodenum. The concentrations of hydrochloric acid in the chyme in the two observations were none and five hundredth normal, and total acidities titrable to phenolphthalein were equivalent to fiftieth normal and twenty-fifth normal. In these observations sodium

4. Cannon, W. B.: Movements of the Stomach Studied by Means of the Roentgen Rays. Am. J. Physiol. 1:359, 1898.

5. Cole, L. G.: Am. J. Physiol. 43:618, 1917.

6. Myers, F. J., and McClendon, J. F.: Note on the Hydrogen Ion Concentration of the Human Duodenum, J. Biol Chem. 41:187, 1920.

bicarbonate solution was poured into the duodenum after the pyloric sphincter had opened and up until the time of its complete closure. The amounts of sodium bicarbonate solution introduced were sufficient to neutralize, in the concentrations in which it was found to be present, a quantity of acid several times greater than the cubic contents of the first portion of the duodenum. For this reason, it is safe to assume that the contents of the duodenum were alkaline in reaction. Then, according to the "acid control" theory, the sphincter should have remained patent as long as the duodenal contents were alkaline. This it did not do, but was observed to close completely. Similar results were obtained in Observation 4. However, absolute evidence that the amount of carbonate solution introduced in the observation neutralized all the acid in the duodenum was not obtained, although but little doubt can reasonably exist but that neutralization was effected. In Observation 3, in which the gastric contents in the region of the sphincter were alkaline, the contents of the duodenum are known positively to have been alkaline. Nevertheless, there was no demonstrable effect on the opening or closure of the pyloric sphincter.

Since the sphincter opens in relation to the advance of each antral peristaltic wave the contents of the antrum are not undergoing a mixing process between the times of opening of the sphincter, as was described by Cannon [4] in cats. For this reason the degree of acidity of the antral contents must be constant during the interval in which the sphincter is closed. That the contents are acid is known. This statement is based on the following facts:

1. The authors, among others, have found the food when first ejected from the stomach into the duodenum to be slightly acid.

2. The contents aspirated from the stomach, after feeding various kinds of meats [7] and after the Riegal test meal [8] of meat and potatoes, are acid and contain free hydrochloric acid.

3. Free hydrochloric acid is constantly present in the stomach after feeding the Ewald test breakfast of bread and water.

From the above it follows that in order for the stimulation of acid in the stomach to open, and in the duodenum to close, the sphincter, there must be assumed the existence of a very delicate balance between the antral and duodenal reflexes in relation to the presence of acid, the duodenal reflex being much the more sensitive.

McClendon and Myers [6] and the authors have found the contents of the first portion of the human duodenum to be either neutral or of low acidity. After emptying itself of food just ejected from the

7. Fishback, H. R.; Smith, C. A.; Bergheim, O.; Lichtenthaeler, R. A.; Rehfuss, M. E., and Hawk, P. B.: Gastric Response to Foods, Am. J. Physiol. 60:174, 204, 222 and 254, 1919.

8. Riegel, F.: Die Erkrankungen des Magens, Wien. 1:123, 1903.

stomach the duodenum will, therefore, be practically neutral in reaction, and in the presence of pancreatic juice possibly alkaline. Then, if acid in the stomach causes the sphincter to relax, the sphincter should become patent when the duodenum empties itself of food. That the sphincter does not relax is demonstrated by the difficulty experienced in pushing barium through the sphincter into the duodenum by palpation of the abdominal wall. In fact, as has already been stated, it is probable that barium can be forced into the duodenum by this means only when the sphincter opens in relation to the advance of an antral peristaltic wave. From this it follows that, if the pyloric sphincter is under "acid control," then the fact that it opens at regular intervals and normally only in a definite relation to the advance of an antral peristaltic wave makes it necessary to assume: (1) either that there is a finely adjusted acid regulatory mechanism in the antral region of the sphincter which produces the proper degree of acidity to relax the sphincter at a fairly exact time in relation to the approach of a peristaltic wave toward the sphincter; or (2) that a comparable mechanism for properly neutralizing the contents of the duodenum must exist. If either or both such mechanisms exist they must be so adjusted as to permit of changes in the rhythm of the time of opening of the sphincter as occurred in one of our observations after pouring alkali onto the antral end of the pyloric sphincter. Furthermore, while the sphincter closes suddenly it remains open an appreciable length of time. Barium flows through the sphincter as an antral peristaltic wave approaches and continues to do so until the wave has spent itself. The sphincter, therefore, does not close as soon as acid enters the duodenum, and the presence of acid we have demonstrated. If acid in the duodenum causes closure of the sphincter then: (1) either the proper degree of acidity must be developed always at a time when an antral wave has spent itself; or (2) a secondary mechanism in some way regulating the reflex must be assumed.

The existence of the various factors outlined are necessary to explain the theory of "acid control" of the sphincter and their existence has not been proved. The assumption of the existence of these factors renders the mechanism of the control of the sphincter exceedingly complex. It is possible that some other less complicated mechanism may exist in man. The latter has been suggested, but not proved, by Luckhardt, Phillips and Carlson.[9] These investigators found a relation existed between the muscular activity of the antrum and the opening of the sphincter.

9. Luckhardt, A. B.; Phillips, H. T., and Carlson, A. J.: Contributions to the Physiology of the Stomach; The Control of the Pylorus, Am. J. Physiol. 50:57, 1919.

CONCLUSIONS

The conclusions which may be drawn from the observations here presented are as follows:

1. Finely divided carbohydrate, protein or fatty foodstuffs begin to leave the normal human stomach within a comparatively few minutes after their initial ingestion.

2. Under normal conditions the human pyloric sphincter opens regularly at the approach of each antral peristaltic wave, allows chyme to pass through into the duodenum during an appreciable length of time, and closes when the antral peristaltic wave has spent itself.

3. The introduction of fortieth normal, twentieth normal or tenth normal hydrochloric acid solutions into the first, second or third portions of the normal human duodenum either produced no effect on the opening of the pyloric sphincter as observed by means of the fluoroscope, or produced effects which were interpreted as the result of abnormal irritation of the duodenal mucosa.

4. Neutralization of the contents of the first portion of the duodenum did not prevent the closing of the pyloric sphincter.

5. The results obtained indicate that acid is not the principal factor controlling the opening and closure of the pyloric sphincter in man.

Palmer-'49
Bib. no. 325

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Gastroenterology 12:879-883. 1949.

GASTROENTEROLOGY
Vol. 12, No. 5, May 1949
Printed in U. S. A.

Case Reports

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ACUTE CORROSIVE GASTRITIS: OBSERVATIONS ON THE GASTRIC MUCOSA FOLLOWING INGESTION OF CONCENTRATED HYDROCHLORIC ACID

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INTRODUCTION

Direct observation of the effect of ingested corrosive poisons on the gastric mucosa is important for both practical and research purposes, but the danger of the gastroscopic procedure following poisoning by strong agents usually precludes early and repeated studies. Late results are well known from roentgen investigations; gastroscopic information has necessarily been fragmentary.

Muñoz Monteavaro[1] has reported the earliest direct studies of the gastric mucosa following ingestion of corrosive substances. Three days after drinking bichloride of mercury, a patient at gastroscopy revealed mucosal congestion, hyperemia, and collections of mucus on the greater curvature and posterior wall of the proximal two-thirds of the stomach. Another patient who had swallowed arsenic seven days previously, showed hyperemia and edema of the proximal halves of the lesser and greater curvatures and the anterior wall; two months later chronic superficial gastritis was found, with areas of mucosal congestion and collections of mucus.

Ronchetti[2] gastroscoped a 17-year old girl 17 days after the ingestion of an unknown amount of caustic red, and found few changes other than generalized hyperemia; peristalsis was active, and the pylorus, although appearing a little small, opened and closed regularly. Henning[3] found hyperemia, edema, hemorrhages, and ulcerations following oxalic acid poisoning. Schindler[4] followed the case of a 19-year old girl who developed erosions, hyperemia and edema after hydrochloric acid poisoning, and eventually saw the return of a normal gastroscopic picture.

It appears from these few reports that corrosive poisons of various types have rather similar effects on the gastric mucosa. This is in contrast to the well-known rather characteristic quality of the surface changes produced by

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many strong liquid agents on the stratified squamous epithelium of the buccal and esophageal mucosa—the necrosis of strong acids, the fixative action of mercuric bichloride, etc.

CASE REPORT

This 23-year old white male, previously well, began drinking heavily one evening, and, after consuming a liter of German wine, purchased a bottle of liquid from a passer-by. In a semi-stuporous state he drank two or three swallows from the bottle. The events from this point were not well recalled by the patient, but he remembered severe burning in the mouth and abdomen. After vomiting many times during the night, he sought hospitalization early the next morning. The suspicious bottle and its contents were recovered, and laboratory analysis revealed concentrated hydrochloric acid.

Upon admission examination, the patient was sluggish and apparently still inebriated to some extent. He vomited continuously, bringing up small amounts of dark brown material and fresh blood. There were necrotic ulcerations over the tongue and uvula. The epigastrium was exquisitely tender but there were no signs of peritoneal irritation and peristaltic sounds were heard. The admission WBC was 26,500 with 94% neutrophils.

Four hours after admission the temperature was 102°, and the course continued to be febrile for 48 hours in spite of large doses of penicillin. For five days the patient complained of severe epigastric pain and substernal fullness, and he vomited intermittently with continued loss of small amounts of blood. From this point there was gradual subjective improvement. He was able to retain a Sippy diet and inert antacids by the sixth day. The diet was advanced rapidly, until by the 40th day after the acid had been swallowed he was able to tolerate a full diet. The burns in the mouth healed by the 10th day, abdominal pain cleared by the 45th day, and substernal discomfort on swallowing by the 50th. Occult blood disappeared from the stools by the 51st. The patient was discharged from the hospital on the 57th day.

X-ray studies were first made on the seventh day. No abnormality was found other than spasticity of various segments of the stomach.

The first gastroscopic examination was made on the tenth day (Figure). The instrument was passed without difficulty and all parts of the stomach were well visualized. The interior configuration of the stomach was normal. The antrum and pylorus were normal anatomically, but no peristalsis was found. There was no spasm during the examination. There was severe mucosal damage, limited to the region of the cardia and the proximal half of the pars media. It was considerably more severe and extensive over the anterior wall than over the posterior. Only the most proximal part of the lesser curvature was affected, the changes fanning out over the walls from the cardia down towards the greater curvature of the pars media. The mucosal damage consisted of a severe hyperemic edema over the regions indicated, with obliteration of all rugae. The edema differed from that which is found gastroscopically in some cases of urticaria in that the surface here appeared rather

hard and dull, rather than succulent and fragile. There were two ulcerations on the anterior wall of the proximal pars media, each measuring an estimated two by two

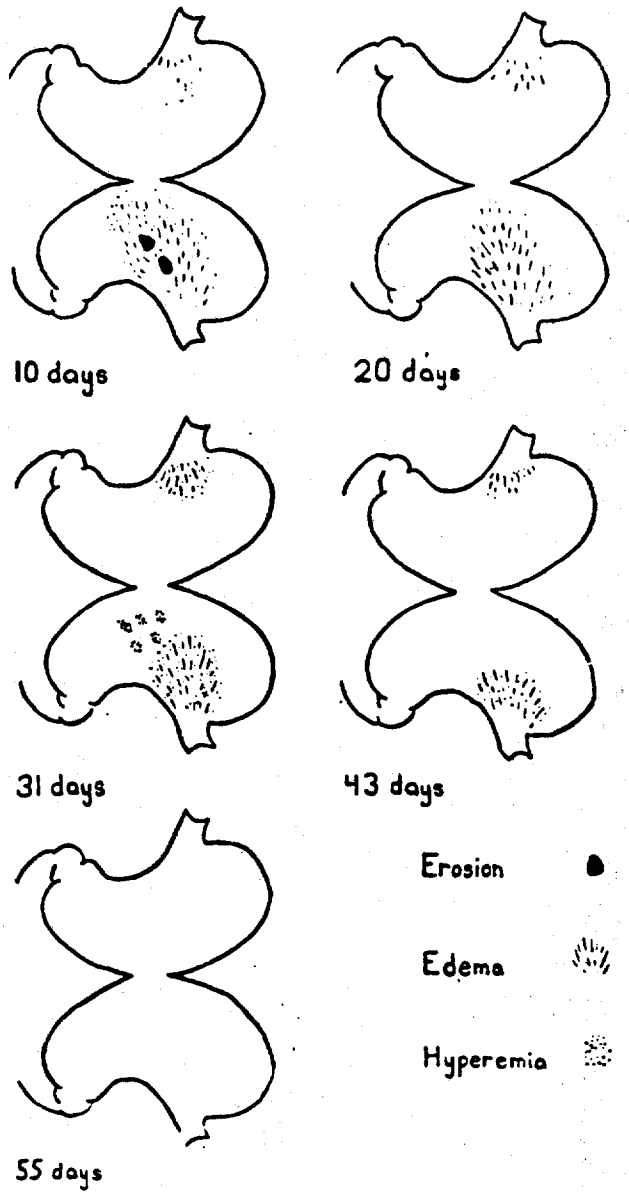

FIGURE. SCHEMATIC REPRESENTATION OF SERIAL GASTROSCOPIC FINDINGS

cm, and each with a dull green-brown necrotic base depressed perhaps a millimeter below the smooth swollen edges. There was no purulent exudate or unusual amount of mucus. There was a small amount of bleeding in the cardia, and the mucous lake was black.

The second gastroscopy on the 20th day showed that the ulcerated areas had healed over and that the mucosa was now everywhere intact. Otherwise, there had been little change in the picture. The distribution of the damage remained as before. The intensity of the hyperemia was striking. The edema was heavy enough to give the anterior wall a fixed appearance. There was no exudate and little mucus. No blood was found.

A gastric analysis with histamine on the 23rd day revealed no free acid and a maximum total acid of 3 CU.

X-ray studies on the 24th day showed no abnormality other than a hyperactive stomach.

On the 31st day, the third gastroscopic examination showed that the damage had begun to clear. The edema had receded on the anterior wall, leaving patches of simple hyperemia in the mid pars media. The mucosa about the cardia was still swollen, but at this time the edema appeared more succulent. There was no blood or exudate. Antral peristalsis was active and coordinated.

The next day gastric analysis with histamine elicited a maximum total acid of 26 and free acid of 16 CU.

On the 43rd day, gastroscopy revealed that improvement had been marked. There was now only mild edema and moderate hyperemia of the cardia. The pars media was normal. Antral and pyloric activity was normal. There was no exudate.

On the 55th day, the stomach was normal at gastroscopy.

DISCUSSION

Gray and Holmes[5] have emphasized that strong alkalies can be expected to cause extensive necrosis of the esophagus, with later cicatricial changes; however, since they are quickly neutralized in the stomach, they have relatively little effect here. On the other hand, strong acids usually affect the esophagus little as compared to the damage they cause in the stomach. As judged by the late effects, much of this damage is exerted on the pyloric region, for pyloric stenosis is a common sequela[5, 6, 7, 8]. In considering the cause for the stenosis which followed ingestion of sulfuric acid by a two year old baby, Vézina[8] concluded that immediate pyloric spasm had concentrated the acid in the pyloric region, with resulting maximum damage at that level. Schulenburg[7] had previously accepted this explanation for the stenosis which quickly followed the swallowing of hydrochloric acid by his patient, on the basis of the experimental radiologic evidence—gained by studies on dogs—presented by Testa[9]. This latter author had followed barium-caustic mixtures along the canine lesser curvature to the pre-antrum, where they caused spasm and delay and, subsequently, most severe damage at the point of delay. Degenhardt and Henderson[6], too, have accepted this explanation. If the experimental evidence can be applied directly to the situation in man, and if, therefore, the clinical theory can be accepted, then rather satisfactory proof becomes available of the physiopathologic validity of the human Magenstrasse.

Gastroscopic information, however, does not lend support to this theory. In no case of corrosive poisoning studied gastroscopically and reported has predilection of the lesser curvature to mucosal damage been found. Rather, the changes have been diffuse over the proximal regions of the stomach, and, in most cases, the antrum and pylorus have been normal. No alternate theory to explain the late pyloric stenotic changes is offered at this time, but it is felt that it is necessary to consider not only the area of greatest concentration of the agent, but also the mere susceptibility of the antral region to noxious influences. Many histologic and clinical studies have indicated that the mucosa of the prepyloric region is "very much more sensitive than that of the corpus to those irritable agents to which the stomach is continually exposed. . ."[10].

CONCLUSIONS

As a result of several examinations one after the other in a case of corrosive gastritis and of a review of the brief literature on the subject, it is concluded that gastroscopic evidence points to an absence of any charactersitic gastric mucosal change resulting from the swallowing of various corrosive agents. The idea of a Magenstrasse is not supported by gastroscopic studies in cases of corrosive gastritis.

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Pathak-'59
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SOME EFFECTS OF INTRODUCING HCl INTO HUMAN STOMACH.

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[Received for publication, September 15, 1958.]

AMONGST the less recognized functions of hydrochloric acid, a normal component of gastric juice, may be mentioned its ability to control the pylorus as well as its own secretion.

Since Cannon put forth his theory of the acid control of the pylorus in 1904, the hypothesis has suffered many reverses. That strong acid closes the pylorus and delays the gastric emptying has been well confirmed (Ivy and McIlvain, 1923; Van Liere and Sleeth, 1940; Quingley *et al.*, 1942), but whether the acid opens the door in mild concentrations has not been substantiated. Besides, HCl possibly regulates its own secretion also. Day and Webster, 1935; Shay, Gershon-Cohen and Fels, 1942; and others have reported that inhibition of the secretion of acid in gastric juice occurs when HCl is introduced into the human stomach.

These two effects of HCl in normal human stomach were studied by the single withdrawal method of Hunt (1951).

METHODS AND MATERIALS.

After coming to the laboratory with empty stomach in the morning, the subject swallowed a modified Levin tube and washed out his stomach after drinking 250 c.c. water. The 'meal' was made up of either distilled water alone or distilled water containing HCl in varying concentrations, tinged with phenol red (60 mg. per litre) used as a marker. In all experiments the volume of the 'meal' was kept uniform, viz. 750 c.c., and its temperature 37°C. It was run into the stomach through the tube to avoid stimulation of taste and other receptors. It took 2½ minutes to empty into the stomach. The subject rested in a chair throughout the experiment. He spat out or aspirated any saliva that was formed. Exactly after 20 minutes the gastric contents were aspirated with the help of a pump (recovery I). This process took about 2½ minutes. Then he washed out his stomach after drinking 250 c.c. distilled water (recovery II). The total volume of the gastric contents recovered was worked out calorimetrically. Acid and chlorides in the 'meal' as well as in 'recovery I' were estimated.

Results of 75 experiments performed in 3 healthy medical subjects are presented here.

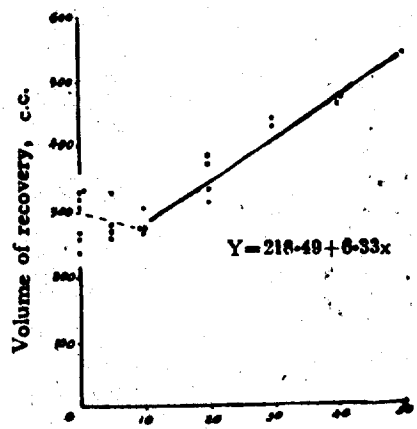
RESULTS AND DISCUSSIONS.

A. Effect of HCl on gastric emptying.—The volumes of the gastric contents remaining in the stomach at the end of 20 minutes are plotted against concentration of HCl in the 'meal' for each subject (Graphs 1, 2 and 3). The data indicate a [illegible] effect of HCl on gastric emptying. There is a progressive retention of gastric

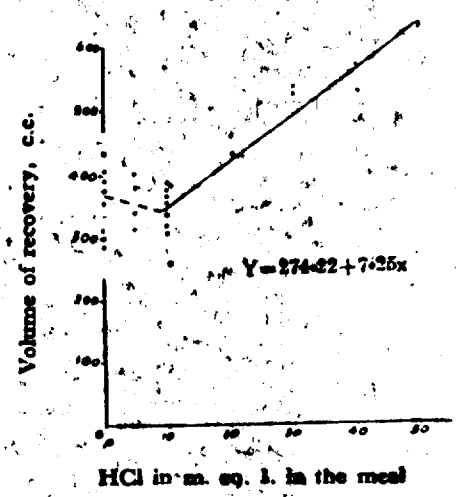
contents after 10 m. eq. l. concentration of HCl in the meal. A straight line is fitted statistically to this data of recoveries for 10 to 50 m. eq. l. HCl in the meal. Higher concentrations of HCl were not tried as the subjects experienced a sense of fullness and warmth with 40-50 m. eq. l. HCl 'meals'.

With lower concentrations of HCl in the meal from 0 to 10 m. eq. l., there does not seem to be any tendency to retention ; on the other hand, the acid seems to favour emptying from the stomach. In Graphs 1, 2 and 3, a dotted line is shown connecting the means of the volumes recovered. It indicates that HCl in milder concentrations facilitates evacuation from the stomach.

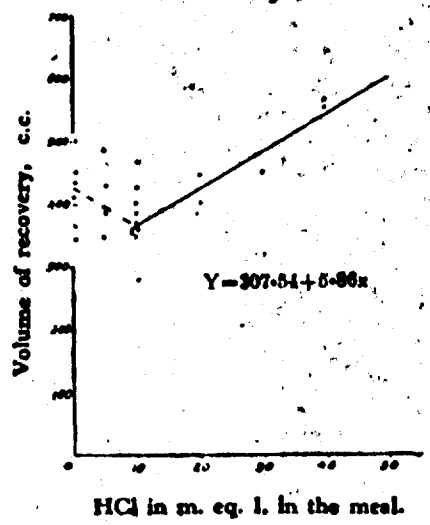
GRAPH 1.



GRAPH 2.



GRAPH 3.



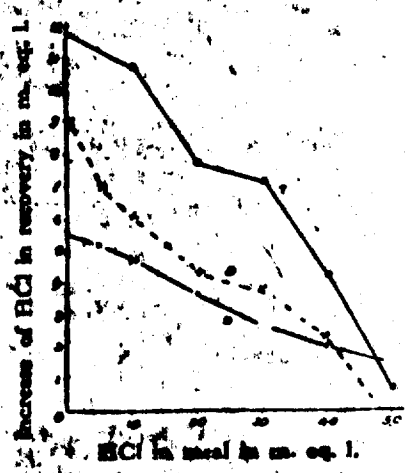
These findings agree with Cannon's observations, who wrote, 'acid retention closely precedes the initial passage of food through the pylorus, that the introduction of acid causes pyloric opening and that delaying the acid reaction causes retention of food in the stomach, in spite of strong peristalsis'. Cannon (1904, 1911), however, recognized that acid could not be the only factor governing the pylorus as the acid control theory could not explain the passage of food in achlorhydrics.

In their experiments on human subjects, Shay and Gershon-Cohen (1934) obtained delay in gastric emptying when relatively higher concentrations of HCl 0·24 to 0·53 per cent, i.e., about 65 to 145 m. eq. l. were introduced into the human stomach. This delaying effect of HCl is said to operate more sensitively from the duodenal side. Frumin (1942) introduced 0·5 per cent HCl into human duodenum and noticed the inhibition of gastric contractions and doubling of its evacuation time. Ivy and McIlvain (*loc. cit.*) had placed 1 per cent HCl into duodenum. This resulted in considerable back pressure leading to vomiting of gastric contents. Thus, evidence regarding the action of strong acid in delaying the emptying is conclusive.

Very few workers have critically examined the influence of weak solutions of of the acid. There is little proof in literature in support of the hurrying effect of mild HCl. Van Liere and Sleath (*loc. cit.*) found that weak solutions of HCl delayed gastric emptying only in achlorhydric subjects. Best (1911), and Barsony and Egan (1925) had noted that weak concentrations of acid had little effect on emptying of the stomach in animals.

B. *Effect on the secretion of acid in the stomach.*—The acid response to varying concentrations of HCl in the meal for each subject differed slightly but the pattern of response was similar for all subjects. The difference between the acid in recovery and in the meal for each subject for varying concentrations in the meal, has been plotted in Graph 4. The first subject shows relatively high, the second moderate

GRAPH 4.



Acid secretory response of 3 subjects (D, P, T.)

and the third a low secretory acid response. Each one shows a decrease in acid contributed by the stomach as the concentration of HCl in the meal rises. The lowest response is obtained with 50 m. eq. l. HCl in the meal and in one subject the difference between the acid in the recovery and the meal is negative. The stronger the acid instilled into the stomach the lesser is the HCl secreted by the organ. The results indicate that HCl regulates its own secretion.

Sokolov (1904) working in Pavlov's laboratory, had noticed that HCl instilled into the stomach diminished the acid secretion in Pavlov's pouch in dogs. In their experiments on human stomach, Day and Webster (1935) obtained inhibition of the flow of gastric juice when 0·25 per cent HCl was introduced into the stomach. Shay, Gershon-Cohen and Fels (1942) found similar depression of gastric secretion in human stomach. The experiments reported here confirm their findings and indicate that such an inhibition is proportionate to the amount of HCl introduced into the stomach.

Summary.

1. Gastric response to varying concentrations of HCl was studied by Hunt's method in 75 experiments performed in three medical subjects.

2. HCl in concentrations up to 10 m. eq. l. seems to favour gastric emptying, whereas with concentrations above 10 m. eq. l. there is a progressively larger retention of the gastric contents. The data support Cannon's theory of the acid control of the pylorus.

3. HCl introduced into the stomach proportionately depresses its own secretion in the stomach.

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Poteshman-'67
Bib No. 347

CORROSIVE GASTRITIS DUE TO HYDROCHLORIC ACID INGESTION*

REPORT OF A CASE

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PYLORIC obstruction resulting from the ingestion of a corrosive substance has been repeatedly noted since the early report of Robert[18] in 1828. The treatment of the stenosis is a surgical problem and most of the articles describing corrosive gastric strictures appear in the surgical literature. There are numerous reports detailing the roentgenographic changes and pointing out the similarity of these changes to those found in cases of carcinoma in the stage of obstruction.[1,3,5,8,12,14,19,21]

Examination of the gastrointestinal tract usually is not obtained until several weeks after ingestion when symptoms of obstruction begin to dominate the clinical picture. A few reports demonstrating the earlier roentgenographic changes before progression to partial or complete stricture are available. Nevin *et al.*[13] reported a case in which a gastrointestinal roentgenographic examination was performed 9 days after ingestion of hydrochloric acid. A polypoid mass due to edematous folds was present in the antrum of the stomach. Later, examination revealed the typical progression to pyloric stricture and obstruction.

The case to be described also afforded an opportunity to examine the gastrointestinal tract at an early stage, at which time a large antral mass due to edema was visualized. In addition, a large ulcer crater due to erosion by the acid substance was demonstrated, making the similarity to an ulcerated gastric neoplasm even more striking in this early stage. The late changes of pyloric and antral stenosis also simulate neoplastic involvement, although there is a marked difference in appearance from the earlier changes.

REPORT OF A CASE

A 47 year old female ingested 4 or 5 ounces of hydrochloric acid (cleaning solution) in an apparent suicide attempt. She was immediately aware of very severe pain and took peptobismol and milk. A few moments later she vomited this material. When first examined, she had injection of the posterior pharynx, was hoarse and had difficulty swallowing. Intravenous fluids were given until 4 days after ingestion, at which time she began to take fluids orally. By 6 days after ingestion she was eating soft foods but still had considerable mucus production from the hypopharynx, hoarseness and dysphagia with substernal pain on swallowing. Mucosal ulceration was present in the oropharynx. Conservative treatment was continued.

Nine days after ingestion, roentgen examination of the esophagus, stomach and duodenum demonstrated a smooth mass in the gastric antrum with a 2 cm. ulcer crater. In addition, the lesser curvature of the stomach extending above the incisura angularis appeared involved by the lesion (Fig. 1). The esophagus appeared normal but the patient did aspirate a small amount of barium into the trachea (Fig. 2).

A repeat gastroduodenal examination to further evaluate the stomach was performed 2 weeks later (23 days after ingestion). At this time, the patient was asymptomatic except for continued severe hoarseness. The appearance of the stomach had changed remarkably in the 2 week period. There was now a severe constriction involving the distal portion of the body and the entire antrum of the stomach, with two small ulcer craters being present in the mid-portion of the constricted area (Fig. 3, *A*, *B* and *C*). There was no significant obstruction and 6 hour delayed roentgenograms showed no residual barium in the stomach. The patient rapidly developed symptoms of obstruction and an antecolic gastrojejunostomy was performed 4 days later (27 days after ingestion).

* From the Department of Radiology, The University of Chicago, Chicago, Illinois.

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At surgery, the wall of the lesser curvature from the cardioesophageal junction to the pylorus was markedly thickened. There was increased vascularity and external signs of inflammation of the serosa. The greater curvature proximal to the pylorus was shrunken and scarred.

DISCUSSION

In a review of the literature up to 1948, Gray and Holmes[5] found 139 cases of pyloric stenosis due to ingestion of acid corrosives. From the report of Boikan and Singer[1] it would appear that the frequency of corrosive pyloric stenosis is greater than these 139 cases would indicate. They reported 5 cases in a period of 1½ years seen at a single institution.

The ingestion of acid substances has a preferential effect on the distal stomach, resulting in progressive stenosis, usually starting 3 to 4 weeks after ingestion. Approximately 20 per cent of patients with pyloric stenosis due to acid ingestion have an associated esophageal stricture.[15,17] Alkaline corrosives have a more marked effect on the esophagus. Heindl[7] reported a 20 per cent incidence of pyloric stenosis in 116 cases of esophageal stricture due to lye ingestion.

Fig. 1. Roentgenogram 9 days after ingestion of hydrochloric acid demonstrates an antral mass due to edematous folds with a large ulcer crater. The lesser curvature also appears to be involved by a mass.

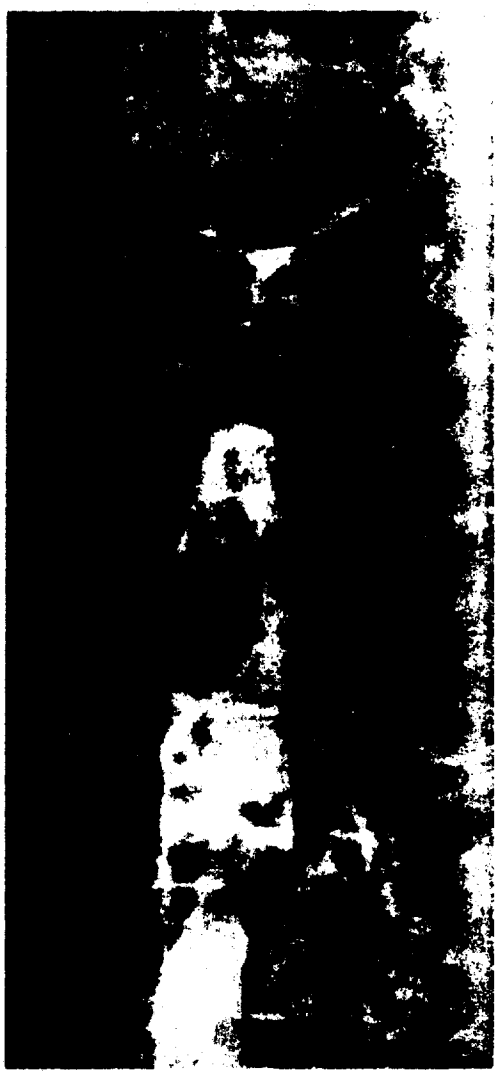

Fig. 2. Aspiration of barium with coating of the larynx and proximal trachea.

The cause of the selective effect of acid on the pylorus is probably related to its rapid passage down the esophagus and along the lesser curvature of the stomach, especially if the stomach is full at the time of ingestion. When the corrosive substance

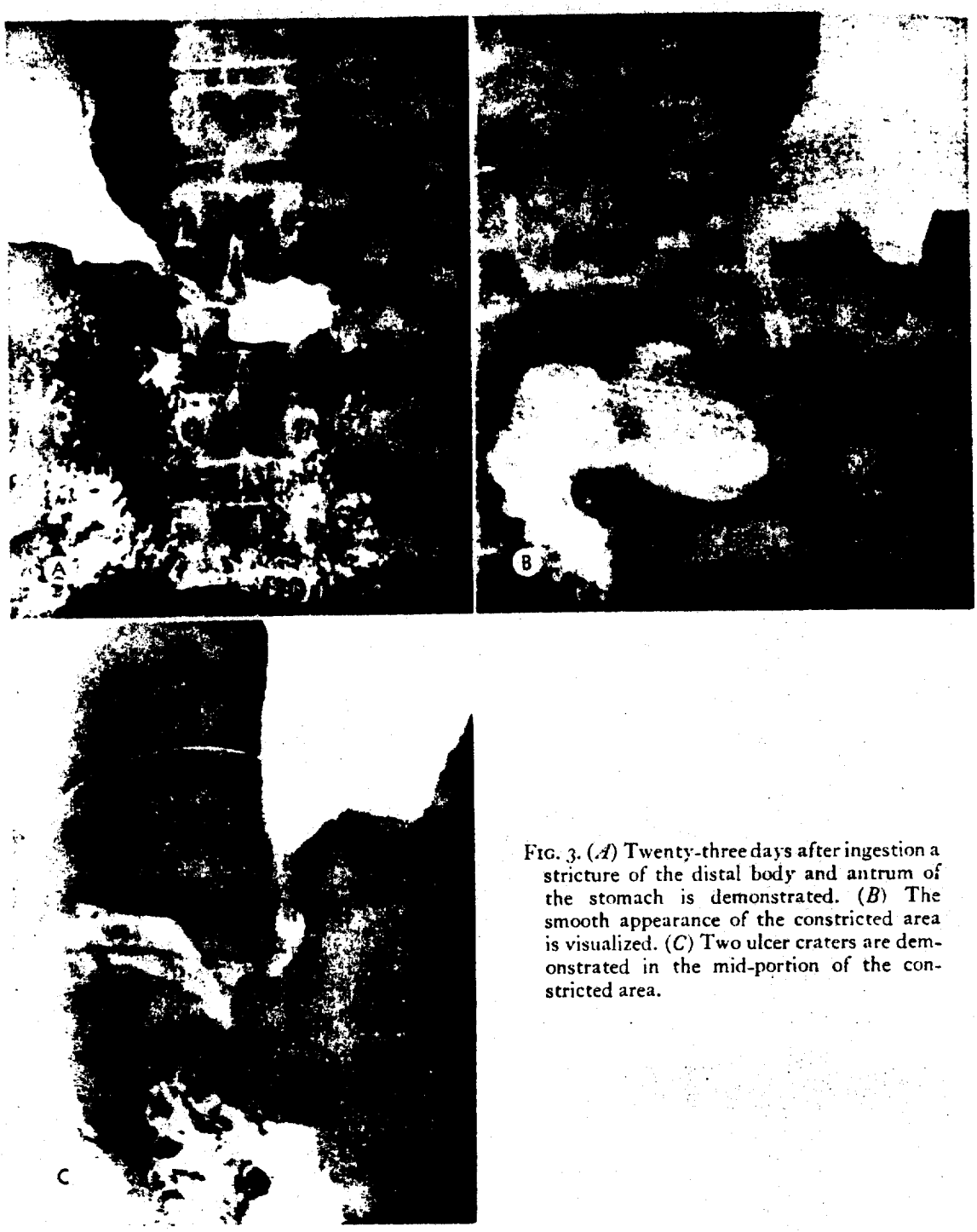

FIG. 3. (*A*) Twenty-three days after ingestion a stricture of the distal body and antrum of the stomach is demonstrated. (*B*) The smooth appearance of the constricted area is visualized. (*C*) Two ulcer craters are demonstrated in the mid-portion of the constricted area.

reaches the antrum and pylorus, tetanic contractions are set up as experimentally demonstrated by Testa.[33] The exposure in this area is, thus, prolonged, resulting in severe changes in the relatively delicate gastric columnar epithelium.

Alkaline substances are more corrosive than acid ones and, therefore, have a greater effect on the relatively resistant esophageal squamous epithelium. In the stomach, however, the normal gastric acidity has a neutralizing effect with re-

sultant sparing of this area.

Corrosive substances which have been reported to cause pyloric stenosis include hydrochloric acid,[6,8,10,12,13,19,20] sulfuric acid,[1,5,21] nitric acid,[1,14,16] acetic acid,[16] trichloracetic acid, carbolic acid, oxalic acid, lye[1] and lysol, ferrous sulfate (feosol),[2,25,26] copper sulfate, bichloride of mercury,[4] formaldehyde,[26,24] clorox,[22] zinc chloride,[12,20] ammonium hydroxide (household ammonia),[4,14] tincture of iodine and chlorine. Hydrochloric acid is the most common agent in cases resulting in pyloric stenosis. Sulfuric acid ingestion is more likely to result in early death due to perforation and gangrene.

Treatment of corrosive pyloric stenosis requires a surgical procedure to bypass the obstruction, usually without gastrectomy. Attempts at conservative therapy, avoiding surgery, are not effective.

SUMMARY

A case of corrosive gastritis due to hydrochloric acid ingestion is reported with demonstration of a corrosive ulcer with surrounding edema in the early stages. In the late stages the typical stenotic lesion with accompanying clinical symptoms of obstruction supervenes. The similarity of both stages to gastric neoplasm is stressed.

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Pritzl-173
Bib No. 351

Poultry Sci. 52(5):1979-1981. 1973.

THE EFFECT OF HYDROCHLORIC, SULFURIC, PHOSPHORIC, AND NITRIC ACIDS IN DIETS FOR BROILER CHICKS[1]

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(Received for publication April 9, 1973)

ABSTRACT To determine the effect of various acids in the diet of chicks, several levels of HNO_3, HCl, H_2SO_4, and H_3PO_4 were fed to mixed sex Indian River broiler chicks.

The birds accepted the diets containing up to 0.27M HNO_3 (16,500 p.p.m. nitrate) without a significant weight loss; higher levels of nitric acid created reduced weight gain, severe death loss, diarrhea, and evidence of dehydration.

The birds responded well to 0.1 M levels of the other three acids, but 0.2 M levels reduced the gain/feed value and weight gain.

It may be concluded that up to 0.1 M levels of HCl, HNO_3, H_2SO_4, and H_3PO_4 can be added to a corn-soybean meal type of diet with no apparent detrimental effect to the broiler-type chick.

POULTRY SCIENCE 52: 1979–1981, 1973

MATERIALS AND METHODS

AT one day of age 480 unsexed Indian River broiler chicks were randomly separated into groups of ten birds each and placed in heated batteries with wire floors. Each treatment was presented to 30 chicks.

On the 1st, 3rd, 10th, 17th, and 24th day of age, group weights and feed conversions were calculated. On the 10th day, those birds which were still alive on the 1.06 M HNO_3 diets were sacrificed and replacement birds substituted for them; the replacement birds had received the control diet and were of the same shipment as the originals.

The basal diet for this experiment is presented in Table 1. Before acid was placed into a diet, an equal volume of water was mixed with the acid. For example, the diet containing 0.06 M of HCl included 30 Kg. of the basal diet plus 63 grams of HCl plus

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TABLE 1.—*Effect of supplemental acids in diets[1] upon total gain, feed consumption and gain/feed ratio*

Acid added to diet	Moles of acid / kg. of feed	% in the diet	pH of feed[2]	pH of feces[2]	Average 27 day total gain(g.)	Gain / feed %	Mortality %
None	0	0	5.9	6.7	535A	59	2
HCl	.06	.21	5.4	5.7	543A[3]	57	0
HCl	.12	.42	5.2	5.7	529A	59	0
HCl	.23	.84	4.6	5.6	494AB	49	0
H_2SO_4	.06	.60	4.8	5.8	508A	57	0
H_2SO_4	.12	1.20	4.4	5.4	504A	58	0
H_2SO_4	.24	2.40	3.6	5.2	407C	52	2
H_3PO_4	.05	.54	5.2	5.7	546A	57	0
H_3PO_4	.10	1.08	5.1	5.7	513A	57	0
H_3PO_4	.20	2.16	4.6	5.5	422CB	55	3
HNO_3	.07	.42	5.3	5.8	525A	57	3
HNO_3	.13	.84	5.0	6.1	521A	61	0
HNO_3	.27	1.68	4.6	5.8	525A	58	0
HNO_3	.53	3.36	3.6	5.3	255D	39	10
HNO_3	1.06	6.72	2.4	5.6	−6.8[4]	−4[4]	73[4]
					−19E	−8	73

[1]The basal diet contained (% basis) the following: 50.55 ground corn, 32 soybean meal (44% protein), 4 menhaden fishmeal, 4 soybean oil, 3 meat and bone meal, 2 brewers' dried yeast, 1.5 dehydrated alfalfa meal, 1.5 dicalcium phosphate, 0.8 ground limestone, 0.3 NaCl, and 0.35 micro-ingredients. The micro-ingredient mix suppled the following per kg. of diet: 6200 I.U. vitamin A, 1100 I.U. vitamin D_3, 105 mg. choline chloride, 33 mg. niacin, 4.4 mg. riboflavin, 14.3 mg. d-pantothenic acid, 4.2 mg. pyridoxine, 2.2 mg. vitamin K, 11 mg. vitamin E, 1.3 mg. folacin, 0.13 mg. biotin, 0.011 mg. vitamin B_{12}, 2.2 mg. thiamine, 50 mg. Mn, 50 mg. Fe, 50 mg. Zn, 5 mg. Cu, 1.5 mg. I, 0.05 mg. Co, and 1 g. methionine.

[2]The pH values represent one observation per treatment when birds were 14 days of age.

[3]Numbers without common letter are different at 1% level of significance.

[4]The two sets of numbers for the 1.06 M HNO_3 diet represent performance of the first group from 0–10 days of age and performance of the second group from 10–27 days of age, respectively.

approximately 350 grams of water with the acid. The diets were stored in closed plastic containers at 20° C. (70° F.) during the course of the experiment.

For the experiment, Baker analyzed Reagent Phosphoric Acid (86.0% pure), J. T. Baker Hydrochloric Acid U.S.P. (37.9% pure), J. T. Baker Sulfuric Acid, Technical (93% pure), and Dupont Nitric Acid Reagent (70% pure) were used.

The pH's listed in Table 1 were determined with a Beckman Zeromatic pH meter. Ten grams of feed or feces (wet weight) were placed in a beaker and 100 ml. of distilled water added. The solutions were allowed to sit after stirring for at least 30 minutes and then the pH of the solution was taken at room temperature.

All weight and gain/feed values were analyzed by Tukey's multiple range analysis.

RESULTS AND DISCUSSION

Up to 0.1 M levels of acid in diets had no detrimental effects on the chicks (Table 1). Where growth restriction was noted, the adverse effect seemed to be elevated as the birds increased in age. The chicks showed no outward signs of impairment, except for the two highest levels of nitric acid.

None of the H_2SO_4 birds appeared to grow as well as the control birds. The 0.05 M level phosphoric acid diet appeared to produce the

heaviest birds at the end of the experiment.

It was quite apparent that the chickens receiving the three lowest levels had little trouble handling the HNO_3 in their diet; the 0.27 M level having 16,500 p.p.m. nitrate in the diet. However, the 0.53 M level caused severe growth restriction; the last week of the experiment the chicks on this diet began to lose weight and their feathers gained a "frizzled" appearance. Diarrhea developed in these birds during the second week of the experiment, and there was a 10% death loss.

The 1.06 M level of HNO_3 in the diet was devastating. Within two days the chicks had severe cases of diarrhea, and by the fourth day, the birds had begun to die. There were only eight birds out of thirty left alive at the 10th day of the experiment. After three days the replacement birds also had diarrhea and began to die. In one of these pens, three birds were killed by two others, indicating a possible increase in cannibalism. The average hematocrit percentage from the original birds on this level of acid was 45, which was higher than the control birds (32–25) and indicates dehydration. These chicks were quite lethargic and had "frizzled" feathers.

In each treatment the pH of feces was above that of the feed (Table 1). No postulate is made as to how the chickens physiologically adjust the pH as much as is indicated in Table 1.

It may be concluded that 0.1 M levels of HCl, HNO_3, H_2SO_4, and H_3PO_4 can be added to a corn-soybean meal type of diet with no apparent detrimental effect to broiler-type chicks.

ACKNOWLEDGEMENTS

We would like to thank Mr. Comp, Ross Cox, Charles Turner, and James Ritta for their help and guidance during the course of this experiment. The following are acknowledged for gratis products used: Hoffman LaRoche, Dow Chemical Company, Merck and Company, Diamond Shamrock Chemical Company, Monsanto Company, Calcium Carbonate Company, and Indian River Inc.

Shambaugh-'34
Bib No. 415

THE EFFECT OF INTRAVENOUS HYDROCHLORIC ACID IN NARCOSIS

EXPERIMENTAL OBSERVATIONS

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Widespread interest has recently been aroused by an enthusiastic report by MacGilvra [1] of the use of intravenous hydrochloric acid as a means of promptly terminating anesthesia both in experimental animals and in man. In the report, successful results are described in terminating ether anesthesia in rabbits, guinea-pigs, rats and monkeys. It was also used successfully in a patient who appeared moribund from the effect of tribrom-ethanol (avertin) anesthesia. The author suggested that the treatment might be equally efficacious in other forms of anesthesia. In view of the obviously great clinical value which a successful and safe method of terminating narcosis would have, it seemed advisable that this procedure should be immediately subjected to further carefully controlled experimental observations.

In a preliminary report [2] we described the results of the intravenous injection of varying amounts of tenth normal hydrochloric acid in dogs which had been anesthetized with ether, tribrom-ethanol and pentobarbital sodium (nembutal). In no case were we able to demonstrate the least effect on the rate of recovery. In spite of these discouraging results, it was felt advisable to continue the observations, using other laboratory animals. Our experiments have now included dogs, guinea-pigs, rabbits, monkeys (Macacus rhesus) and baboons (Papio papio). All the experiments were carefully controlled by simultaneous observations on untreated animals. In each case the experimental procedure was as follows: Deep narcosis was induced in two animals, following which one was given one or more intravenous injections of tenth normal hydrochloric acid, while the other was untreated or, in certain cases in which it was felt that the stimulation incident on the venipuncture might hasten the recovery rate, the control animal was merely pricked with a needle. The rate of recovery from narcosis was determined, as accurately as possible, by noting the time of the return of the wink reflex, voluntary movements of the legs,

TABLE 1.—*Effect of Hydrochloric Acid in Ether Anesthesia*

Animal	Weight, Kg.	HCl N/10, Cc.	Reflexes Present	Legs Move	Attempts to Stand	Stands
Dog C25	23.6	3	1½ min.	6 min.	10 min.	16 min.
Z18*	20.4	..	1	4	7	9
Z18	20.4	3 3 3	1	4	7	12
C25*	23.6	..	1½	5	9	13
Baboon (Papio papio)						
A142	3.5	2	0	1	1½	3
A143*	4.5	..	0	1	1½	3
A143	4.5	2	0	1½	2½	4
A142*	3.5	..	0	1	1½	3
A143	4.5	2	0	1½	2½	4
A143*	4.5	..	0	1	1½	3
A142	3.5	2	0	1	1½	3
A142*	3.5	..	0	1	1½	3
Rabbit						
C1	2.4	2	Died			
C2*	2.35	..	2½	3	4	5
C3	1.5	2	½	2	2½	3½
C3*	1.5	..	1	2	2	4
C2	2.35	2	Died			

* Control.

TABLE 2.—*Effect of Hydrochloric Acid in Pentobarbital Sodium Anesthesia*

Animal	Weight, Kg.	Dose, Gm.	HCl N/10, Cc.	Reflexes Present	Legs Move	Attempts to Stand	Stands
Dog							
H79	26.4	0.78	2 2 2 2 2 2	80 min.	..	..	16 hrs.
156*	31.4	0.98	..	80	..	..	6 hrs.
J57	21.0	0.63	100 100 100 50	No change; remained in deep anesthesia until killed two hours later			
J40*	20.5	0.62	...	Same as above			
Monkey (Macacus rhesus)							
C127	2.5	0.08	2	..	..	..	12 hrs.
C127	2.5	0.08	..	..	..	..	12 hrs.
Rabbit							
C3	1.5	0.10	2	10 sec.	10 sec.	15 sec.	30 sec.
C4*	1.4	0.10	1‡	10 sec.	10 sec.	15 sec.	30 sec.
C3†	1.5	0.10	2	55	..	..	2 hrs.
C4*†	1.4	0.10	..	60	..	..	2 hrs.
Guinea-pig							
C1	0.43	0.032	2	Died			
C2*	0.42	0.032	..	..	..	..	8 hrs.

* Control.
† Pentobarbital sodium given five minutes after cessation of previous observation.
‡ Subcutaneously.

12. Quoted by Plass, Hesseltine and Borts.[13]
13. Plass, E. D.; Hesseltine, H. C., and Borts, I. H.: Monilia Vulvovaginitis, Am. J. Obst. & Gynec. **21**: 320-334 (March) 1931.
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From the Laboratory of Surgical Research, Harvard Medical School.

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attempts to stand, and finally the ability to stand unassisted. The results are shown in the accompanying tables. The time intervals noted were taken from the withdrawal of the anesthetic in the case of the ether and from the point of deep narcosis in the case of tribrom-ethanol and pentobarbital sodium. The hydrochloric acid was injected at the onset of the timing, and when more than one dose was given these followed at varying intervals of from five to thirty minutes.

In the case of ether anesthesia, as is noted in table 1, the recovery rate was quite rapid even without any form of treatment. This was especially true in the rabbits,

TABLE 3.—*Effect of Hydrochloric Acid in Tribrom-Ethanol Anesthesia*

Dog	Weight, Kg.	Dose, Gm.	HCl N/10, Cc.	Reflexes Present	Legs Move	Attempts to Stand	Stands
Q32	11.0	0.125	5 20 20 20	25 min.	35 min.	50 min.	60 min.
B33*	11.4	0.125	..	30	35	60	65
Q34	26.0	0.135	5	1	6	10	20
Z7*	24.4	0.135	..	(never abs.)	3	10	15

* Control.

guinea-pigs and baboons, so it would necessarily be very difficult to establish the value of any form of therapy in these animals. As far as we could judge, however, the acid in no case shortened the recovery period. Two of the rabbits were inadvertently overanesthetized, and in these the injection of the acid within a few seconds of the cessation of respiration was without effect. Pentobarbital sodium (table 2) was selected as representative of the barbiturates and was administered intraperitoneally, in a 3 per cent solution in physiologic solution of sodium chloride. The doses in most cases approximated 30 mg. per kilogram. As in the case of ether, we found the acid to be entirely without effect even when given in massive dosage totaling in one case 350 cc. The observations in tribrom-ethanol narcosis (table 3) were likewise completely negative.[3]

SUMMARY

Observations on guinea-pigs, rabbits, dogs, monkeys and baboons indicate that the intravenous injection of dilute hydrochloric acid is without effect as a means of shortening the recovery period from ether, pentobarbital sodium and tribrom-ethanol narcosis.

3. W. deB. MacNider, in the course of his extensive investigations in dogs on the effect of intravenous hydrochloric acid on the kidneys, observed no change in depth of anesthesia induced by chloroform and morphine (personal communication to the authors).

Shambaugh -'34
Bib No. 416

Proc. Soc. Exp. Biol. and Med. 31(6);711-712. 1934.

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Effect of Intravenous Hydrochloric Acid upon Narcosis in Dogs.

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In view of a recent report by MacGilvra,[1] describing a remarkable effect of the intravenous administration of hydrochloric acid as a means of terminating anesthesia, it seemed advisable that further experimental studies be carried out in order to establish more definitely a possible clinical application. MacGilvra, repeating observations made by Moorman, terminated ether anesthesia in rabbits guinea pigs, rats and monkeys by the intravenous injection of small amounts of N/10 hydrochloric acid. MacGilvra reported the clinical application of this method for terminating anesthesia (Palinaesthesia) in a patient under "Avertin."

Our observations were made on 8 dogs, using "Nembutal" intraperitoneally, "Avertin" by rectum and ether by inhalation. It was found impracticable to use chloroform because of the extremely rapid, spontaneous recovery from this drug in the dog. Each observation was made on 2 dogs, simultaneously, one of which was given the intravenous hydrochloric acid. Each pair of dogs was matched, as closely as possible, in weight and breed. Considerable difficulty was encountered in determining the depth of narcosis at the various intervals during the observations, so an arbitrary set of criteria was adopted. In each case an approximately equal degree of deep anesthesia was induced, and the rapidity of recovery gauged by noting the time of appearance of wink reflex, spontaneous movements of the forelegs, attempt to stand, and finally, the ability to stand unassisted. The dose of hydrochloric acid varied from a single injection of 3 cc. of N/10 aqueous solution to repeated injections totalling 65 cc. of the same solution. No untoward effects were noted. The results of our observations are shown in the accompanying table.

It is apparent, that in no case did hydrochloric acid accelerate recovery from anesthesia in the dog. On the other hand, in almost every instance, the animal receiving this medication recovered more slowly than the control. It should be stated, however, that the animal selected for the hydrochloric acid was apparently the deeper anesthetized, so that it was not definitely established that the hydro-

[1] MacGilvra, W. V., *Harv. Dent. Rec.*, 1934, 8, 1.

TABLE I.

Dog No.		Wt.	Anesthesia	HCl-N/10	Return Reflexes	Legs Move	Attempt to Stand	Stands
		Kilos		cc.	Minutes			
C25		23.6	Ether	3	(100 sec.)	6	10	15
Z18	C	20.4	"	—	1	4	7	9
Z18		20.4	"	3	1	4	7	12
				3				
				3				
C25	C	23.6	"	—	1½	5	9	13
Q32		11.0	Avertin (.125 gm.)	5	25	35	50	60
				20				
				20				
				20				
B343	C	11.4	" " "	—	30	35	60	65
Q34		26.9	" (.135 gm.)	5	1	6	10	20
Z7	C	24.4	" " "	—	(not abs.)	3	10	15
I179		26.4	Nembutal (.78 gm.)	2	80	—	—	16 hrs.
				2				
				2				
				2				
				2				
				2				
I56	C	31.4	" (.98 gm.)	—	80	—	—	6 hrs.

C equals Control.

chloric acid inhibited recovery. Although our observations have shown no effect comparable to that reported by previous observers, further studies on the other laboratory animals are being made.

Am. J. Digestive Diseases Nutr. 6:361-363. 1939.

Shay-'39

Bib No. 419

The Absorption of Hydrochloric Acid by the Human Stomach*

By

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SEVERAL years ago while studying the problem of duodenal regurgitation in man in relation to the control of gastric acidity, we (1) used in some of the experiments a hydrochloric acid gastric meal. We were impressed by the rapidity with which the acidity of the test meal decreased. Believing then that the rapid changes observed were due neither to dilution nor to neutralization, we wondered whether they might not represent an absorption of hydrochloric acid by the stomach. To the acid meal we added various indicator substances which we hoped would not be adsorbed or absorbed by the stomach mucosa, and attempted, at that time, to study this mechanism further. Unfortunately, we were unable to find an indicator that was suitable and were forced to leave our suspicion without experimental support.

In 1933, Teorell (2) advanced a modified version of the mechanism suggested by us. From studies in the cat, he believed that hydrochloric acid diffused out of the stomach, through the mucosal wall, while neutral chloride passed into the gastric cavity. He believed that there normally exists across the gastric mucosa an exchange of hydrogen-ion from the gastric juice and sodium-ion from the tissue fluid or blood. Latterly, Wilhelmj, O'Brien and Hill (3), studying secretion in whole stomach pouches in dogs did not find support for an acid absorption mechanism.

In a recent splendid survey of the factors which reduce gastric acidity, Hollander (4) questions the likelihood of HCl absorption. This on the basis that such a process could in no way account for the low acid values at the beginning of secretion although he does admit that it may explain the post maximal drop in acidity.

In fundic pouches in dogs in which the secretion was retained for considerable periods by means of a sphincter action on the mouth of the pouch, Hollander failed to find a significant diminution in acidity. While these experiments supplied definite evidence that acid was not absorbed by fundic pouches, Hollander recognized the possibility that other parts of the gastric mucosa might do so. The question of gastric absorption of acid remained incompletely answered.

In 1934, we (5) showed that adequate duodenal stimulation in man may close the pylorus completely and thus stop gastric evacuation for variable periods. This method, coupled with the introduction of one suitable to the determination of phenol red in gastric contents by Hollander, Penner and Saltzman (6), prompted us to reexamine the question of the gastric absorption of hydrochloric acid. While phenol red was studied by Hollander, et al, as a dilution indicator and as such possessed most of the ideal requisite characteristics as first suggested by Gorham (7), we employed it rather as a measure of pyloric closure, recovering 100% of the ingested dye from the stomach at the end of the test period. That phenol red may be so employed is supported by the recent work of Penner, Hollander and Saltzman (8) who reported that this dye is not absorbed from the human stomach, is not altered chemically by its contact with the gastric secretions, and does not preferentially stain the mucous membrane to a significant degree. Our own experiments in which we have used phenol red as an indicator fully confirm their results. The principle of the method of phenol red determination of Hollander and his associates consists briefly in centrifugation of the recovered gastric specimen, alkalinization with CaO, treatment with NaOH and ZN SO_4 to precipitate proteins and bile, centrifugation, and treatment of the supernatant fluid with Na_3 PO_4 to remove excess Zn and to adjust the pH to about 11-12, a value at which the phenol red is entirely on the alkaline side. Comparison is then made with a standard phenol red solution. We found that colorimetric comparison was simplified by introducing a green filter Wratten 74.

EXPERIMENTAL METHOD

Patients without gastric complaints who showed anatomically normal stomachs by X-ray were selected. Two groups were chosen, those with a normal gastric secretory response to the Ewald meal and others who were anacid with such a meal. These were thoroughly tube-broken by several intubations before the studies were undertaken. After an overnight fast, the stomach was intubated with two tubes. One was passed into the duodenum in the usual manner while the other remained in the stomach. Olive oil was instilled through the duodenal tube at the rate of 50 drops per minute. The stomach was then completely emptied in all positions of its fasting contents through the gastric tube. Through this tube was then instilled 200 cc. of a solution of approximately 0.5% or 1% HCl in distilled water containing 2 mgms. of phenol red per 100 cc. Thirty minutes after the gastric instillation, the stomach was emptied completely in all positions through the gastric tube. The stomach was then lavaged twice with 200 cc. portions of distilled water and emptied completely after each lavage.

A portion of the test meal, the recovered gastric contents, and all the washes were examined for pH with a Beckman pH meter; for total chlorides by the Wilson and Ball (9) method, and for phenol red as indicated above. To read very dilute solutions of the phenol red, we found useful the application suggested by Wilhelmj (10); namely, adding the unknown to a portion of the standard solution and reading against

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TABLE I

Specimen	Vol. cc.	Free Acid mEq./1	Total Acid mEq.	pH	Conc. Chloride mEq./1	Total Chloride mEq.	Conc. Dye mgm./100 cc.	Total Dye mgm.
Test Meal	200	133.1	13.31	0.98	133.1	26.62	2.00	4.00
Recovered Meal	230	110	12.0	1.09	128.7	29.60	1.63	3.74
First Wash	200	0	0	5.64	0	0	0	0
Recovered First Wash	210	not done	not done	2.22	10.7	2.25	0.11	0.23
Second Wash	200	0	0	5.64	0	0	0	0
Recovered Second Wash	205	not done	not done	3.01	3.5	0.72	0.04	0.08

SUMMARY

Total Chloride Test Meal	26.62 mEq.
Total Chloride Recovered	32.57 mEq.
Total Chloride Added by Stomach	5.95 mEq.
Percentage Increase Total Chloride	23%
Chloride Concentration Fasting Gastric Contents	81.7 mEq/1.

Not only did absorption not occur with this concentration of acid 133.1 mEq/1. (0.48%) but actual secretion must have occurred because of the increase in chloride recovered. This increase could not represent fasting contents left behind because it would have required 73 cc. of the fasting contents to have supplied the additional chloride.

Total Dye Test Meal	4.00 mgms.
Total Dye Recovered	4.05 mgms.
Difference	0.05 mgms.
Error	1.3%

If we consider the steps involved both in recovery of the samples and the determination of the dye in each sample, the above is a very acceptable experimental error.

a similarly diluted standard. Titration of free acid was done in the usual manner.

We have repeatedly obtained results in both normal acid and anacid patients as illustrated in Tables I and II.

DISCUSSION

Our data show that hydrochloric acid concentrations of approximately 0.5% or 1% are not absorbed by the human stomach when left in contact for periods up to 30 minutes. Considering the speed of concentrated glucose absorption by the stomach (11) in the same period of time, we believe the trial period adequate.

Our data indicate also the acidity required to inhibit gastric secretion completely. There is a concept that the introduction of approximately 0.1 normal (0.36%) solution of hydrochloric acid into the stomach will always completely inhibit the secretion of acid chloride during a thirty minute test period. This has not been confirmed by the work of Apperly and Norris (12) or of Wilhelmj, O'Brien and Hill (13). More recently, Welch and Comfort (14) found that following such a test meal, hydrochloric acid was definitely secreted in 65% of their cases and was probably secreted even in a great majority of their remaining cases. Our own experiments show that test meals of concentration as

TABLE II

Specimen	Vol. cc.	Free Acid mEq./1	Total Acid mEq.	pH	Conc. Chloride mEq./1	Total Chloride mEq.	Conc. Dye mgm. 100 cc.	Total Dye mgm.
Test Meal	200	275.3	27.53	0.68	275.3	55.06	2.00	4.00
Recovered Meal	225	210	23.2	0.76	232.3	52.30	1.66	3.74
First Wash	200	0	0	5.72	0	0	0	0
Recovered First Wash	205	not done	not done	2.11	11.6	2.38	0.07	0.14
Second Wash	200	0	0	5.72	0	0	0	0
Recovered Second Wash	205	not done	not done	3.27	5.2	1.07	0.34	0.68

SUMMARY

Total Chloride Test Meal	55.06 mEq.
Total Chloride Recovered	55.75 mEq.
Total Chloride Added by Stomach	.69 mEq.
Percentage Increase Total Chloride	1.3%

In this experiment one may conclude that with the concentration of acid 275.3 mEq 1. (1.0%), no secretion or absorption occurred.

Total Dye Test Meal	4.00 mgms.
Total Dye Recovered	3.96 mgms.
Difference	0.04
Error	1%

The recovery of dye in both charts is adequate evidence that the pylorus had been effectively closed during the test period.

high as 0.5% hydrochloric acid failed to prevent gastric secretion for a period of 30 minutes. In fourteen such experiments the recovery of chloride ranged from 15% to 23% above the total chloride content of the test meal. In 12 experiments in which approximately a 1% acid meal was used, gastric secretion was completely inhibited for the duration of the experiments.

SUMMARY

We were able to study the question of HCl absorption in man by inducing sufficient duodenal stimulation to arrest gastric evacuation and by using phenol red as an indicator of that arrest. This was possible because phenol red in concentration of two milligrams per 100 cc. is not absorbed by the human stomach. Hydrochloric acid in concentrations of approximately 0.5% or 1% is not absorbed by the stomach when such solutions are in contact with it for periods up to 30 minutes. Hydrochloric acid meals of 0.5% concentration do not completely inhibit gastric secretion, while 1% meals do cause complete inhibition.

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Bib no. 430

J. Biol. Chem. 47:315-319. 1921.

THE EFFECT OF HYDROCHLORIC ACID INGESTION UPON THE COMPOSITION OF THE URINE IN MAN.

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(Received for publication, May 12, 1921.)

The data presented herewith are in a way an extension of an experiment reported in 1915 which concerned the effect of the administration of hydrochloric acid on the excretion of Na, K, Mg, and NH_3 in the urine and feces of dogs (1). Increases in the amounts of all these substances were noted in the urine. The augmentation was especially noteworthy in the case of ammonia and almost negligible in the cases of calcium and magnesium. However, the data showed the calcium and magnesium content of the feces to be increased while sodium and potassium were unaffected.

Not long after the above results were published a paper by Givens (2) appeared in which the author concluded that his data failed to demonstrate any unequivocal effect of hydrochloric acid on the calcium and magnesium content of the feces of dogs. In view of the conflicting data it must be admitted that the question is still an open one but an examination of the respective sets of data will show that those of Givens show considerable lack of evenness.

In Table II on Diet B, Dog M does show a higher Ca excretion during the acid period than during the control period but instead of making the obvious interpretation that the acid was the cause Givens offers the explanation that "it seems more plausible to assume that in the fore period . . . . the animal was trying to store up some lime after its depletion by the former low lime régime." "Former" refers to a previous experimental period. Neither is it at all obvious why, in the same table, the

excretion of Ca on the highest Ca diet falls short of the quantity excreted during the preceding acid period. This applies to the experiment with Dog N as well, but in this instance the Ca excretion during the period of highest Ca intake is even lower than that in a period of lower lime intake.

The problem as far as the dog is concerned has some rather difficult aspects. In order to overcome the difficulty resulting from the impossibility of separating the feces of the experimental periods sharply it is necessary to have long experimental periods. The diet must, of course, be uniform throughout the experiment and consequently of a nature so attractive that it will at no time be refused. As far as the dog is concerned this means that meat must be the principal constituent. Meat, however, is well suited to defeat the object desired since the ammonia resulting from its metabolism at once becomes available for the neutralization of any acid administered. Another possible difficulty presents itself in trying to determine where any bases excreted in response to acid administration will be found. It is not necessarily true that they need be in the feces corresponding to the day on which the acid was administered. Conceivably, they may be found in the feces of an earlier day depending upon what part of the alimentary tract is concerned with the excretion.

The present communication deals with the effect of hydrochloric acid ingestion upon the urinary composition in man. Advantage was taken of the experience gained in the earlier experiments and the diet so arranged as to make any variations as prominent as possible. It was ample calorically, but low in protein and sodium chloride. The components were rice, French fried potatoes, bananas, wheat bread, jam, butter, apple pie, and figs. None of the food was salted. The quantities were as uniform from day to day as careful measurement could make them and the only variation throughout the 7 days the diet was adhered to consisted in the ingestion of 3.65 gm. of hydrochloric acid in the form of tenth normal acid daily during the last 3 days. It was divided into three approximately equal portions and was drunk just before meals.

Analytical Methods.

For the estimation of Ca and Mg the procedure developed by McCrudden (3) was employed. Na and K were first isolated as the mixed sulfates and the potassium was then separated as the cobaltic nitrite compound. The yellow precipitate was then oxidized with a standard permanganate solution. The details of the process were those described by Drushel (4). Ammonia was

TABLE I.

Individual.	Day of diet.	Total N.	Chlorides as NaCl.	NH_3	Na as NaCl.	K as KCl.	Ca	Mg	H_3PO_4	Ph
A. C. M.	3	6.85	4.22	0.294	3.56	4.61	0.058	0.104	1.11	6.70
	4	6.59	5.70	0.256	2.55	3.80	0.075	0.115	1.18	6.75
	5	7.95	10.47	0.559	5.19	8.74	0.090	0.181	1.64	6.28
	6	7.34	9.36	0.663	4.09	6.26	0.090	0.159	1.74	5.27
	7	6.97	11.69	0.731	4.11	7.17	0.113	0.161	1.69	5.05
R. L. S.	3	6.27	4.38	0.399	3.60	2.70	0.072	0.106	0.85	5.86
	4	6.16	5.03	0.300	3.70	4.10	0.100	0.094	0.97	6.44
	5	6.42	8.73	0.515	4.60	5.26	0.141	0.142	1.27	5.89
	6	6.29	9.76	0.730	4.29	5.96	0.123	0.135	1.53	5.05
	7	6.29	9.63	0.758	3.29	5.90	0.171	0.122	1.64	5.00

TABLE II.

	A. C. M.	R. L. S.
NaCl equivalent of increased NH_3 excretion	1.294	1.091
NaCl " " " Na "	1.41	0.41
NaCl " " " K "	2.49	1.81
NaCl " " " Ca "	0.090	0.172
NaCl " " " Mg "	0.282	0.161
NaCl " " " H^+ "	0.340	0.271
Total	5.906	3.915

determined by aeration, chlorides by the Volhard-Arnold method, and phosphates by titration with uranium acetate. The hydrogen ion concentration was measured electrometrically.

The effect of the acid on some of the urinary constituents was as given in Table I.

The sodium chloride equivalent of the 3.65 gm. of hydrochloric acid taken daily is 5.83 gm. If the sodium chloride equivalents

of the increased NH_3, Na, K, Ca, Mg, and H^+ concentrations are calculated the results are as given in Table II.

The changes in H ion concentration are taken into account here for the following reason. The pH of the foreperiod shows that there must have been practically equal quantities of primary and secondary phosphates present. The pH of the acid period shows that practically all of the phosphate must have been present in the primary form. Consequently, while the conversion of the secondary phosphate into primary does not involve any change in the amount of phosphate excreted it does signify that a portion of the hydrochloric acid has been neutralized and must be taken into account in the attempt to account for the hydrochloric acid ingested.

If it be assumed that the increased phosphoric acid excretion is due to the conversion of disodium phosphate of the blood and tissues into monosodium phosphate, then for every atom of sodium excreted as sodium chloride which came from the disodium phosphate in question there will have been excreted an extra atom of sodium in the form of monosodium phosphate. Hence from the sum of the sodium chloride increases given above one atom of sodium for every extra molecule of phosphoric acid must be subtracted.

	A. C. M.	R. L. S.
NaCl equivalent of increased NH_3, Na, K, Ca, Mg, and H^+ excretion	5.906	3.915
Na excreted as NaH_2PO_4 and not indicative of acid neutralization	0.328	0.340
NaCl equivalent of acid neutralizing factors	5.578	3.575

In the case of A. C. M. 96 per cent of the ingested acid is accounted for while in the case of R. L. S. the fate of only 61 per cent is apparent. Both of these percentages are undoubtedly influenced by physiological factors, the control of which was impossible, and it is probably true that the mean of the two would approach the true average value more closely than does either of them alone. This would leave about 22 per cent of the acid to be accounted for. The results of similar experiments on

the dog mentioned at the beginning indicate that increased excretion of calcium and magnesium by the intestine may be a factor, but no data are available in the present instance to indicate the extent to which this may be true.

If the bases of the body are drawn upon in any condition of acidosis in the same way that they have been drawn upon by the hydrochloric acid in the present experiments then it is interesting to see how the drain could be met to the best advantage. A simple calculation based upon the figures of A. C. M. in Table II shows that for every 3.65 gm. of hydrochloric acid (which is equivalent to 10.4 gm. of β-hydroxybutyric acid) there would be required in order to balance the increased NH_3 formation and to meet the loss of K, Na, and H_3PO_4, 3.70 gm. of $KHCO_3$, 0.98 gm. of K_2HPO_4, and 3.88 gm. of $NaHCO_3$.

The calcium excretion *via* the urine is affected to a negligible extent but if the fecal excretion is affected in man to the same extent that it is in the dog as shown in the experiment previously referred to (1) then the above materials should be supplemented by a small quantity of some suitable calcium compound. It may be, however, that under conditions of alkali administration the effect on calcium excretion would be of no consequence.

CONCLUSIONS.

Data are presented which show that the ingestion of hydrochloric acid causes an increased excretion of potassium, sodium, ammonia, phosphoric acid, and hydrogen ions.

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New Engl. J. Med. 254:981-986. 1956.

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Bib no. 431

CORROSIVE (ACID) GASTRITIS*

Management of Early and Late Cases

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THE American literature contains comparatively few reports of cases of corrosive gastritis, of either the acute or the chronic variety. Because few patients survive the initial insult for more than twenty-four hours, the concept of the management of such cases is somewhat nebulous. Some of the reports in the literature deal primarily with the late sequelae of corrosive poisoning. Very few patients have survived the initial insult long enough to be followed under medical supervision until the late sequelae of pyloric obstruction appeared. Some steps in the management of such cases have been referred to in a previous publication by one of us (F.S.) and will be reiterated subsequently.

The 4 cases of corrosive poisoning reported below (1 after operation) clearly illustrate the steps in adequate and inadequate management.

CASE REPORTS

CASE 1. M.D., a 36-year-old woman, entered Cook County Hospital on December 8, 1953, because of hematemesis.

Eleven days previously, just after her Thanksgiving dinner, she had become depressed because of her husband's desertion, and she had ingested 59 ml. (2 ounces) of a material that she thought was "lye." (It was later established that she had ingested "Snobol," a commercial disinfectant containing hydrochloric acid.) She immediately fainted and fell to the floor. Her relatives found her lying on the floor, rushed her to a hospital, where her stomach was "pumped." She was released from the hospital the next day, at her own request, despite the fact that she remained very depressed, complained of burning of her throat and stomach and vomited both liquid and solid food. At home she continued drinking beer excessively despite her symptoms. On the day before admission she drank very heavily and this was followed by emesis of about 1 liter of bright-red blood. On the day of admission she vomited another 500 ml. of blood and was therefore admitted as an emergency to Cook County Hospital.

Physical examination revealed a well developed and well nourished, pale woman complaining of substernal pain. Examination of the oropharynx was negative. The lungs were clear to percussion and auscultation; the heart was of normal size and configuration, with rapid heart tones but without murmurs or other abnormal sounds. The abdomen was soft and nontender, and no masses were palpable.

The temperature was 99°F. by mouth, the pulse 132, and the respirations 26. The blood pressure was 82/68.

The examining physician made a diagnosis of corrosive esophagitis due to lye ingestion, with esophageal varices and other causes of massive upper gastrointestinal hemorrhage to be considered.

The patient was treated with whole-blood transfusions, infusions of 5 per cent dextrose in physiologic saline solution, antibiotics and milk and olive oil alternately by mouth. Phenobarbital and atropine were given by hypodermic injection. She improved rapidly and was subsequently placed on a bland diet, which she tolerated quite well. On the 6th hospital day a fluoroscopic examination with Lipiodol and then barium revealed a normal esophagus. According to [illegible] was discharged on the 7th day to the gastrointestinal outpatient clinic on a bland diet and antispasmodics.

She was next seen 3 weeks later by the clinic physician with complaints of a weight loss of 13.6 kg. (30 pounds), weakness and inability to keep solid or liquid food in her stomach. She stated that she vomited either immediately or 2 or 3 hours after eating. Because of these symptoms she was readmitted to the hospital.

Physical examination revealed an alert, co-operative woman, with evidence of weight loss. Examination of the head, chest and abdomen was negative. The physician's impression was esophageal stricture secondary to lye ingestion, with esophageal varices, diaphragmatic hernia, alcoholic gastritis, peptic ulcer and hysteria to be considered.

The pulse was 72 and regular, and the respirations 18. The blood pressure was 114/70.

Urinalysis was negative for sugar, albumin, acetone, urobilinogen and bile. The blood Kahn test was negative. Examination of the blood showed a red-cell count of 4,200,000 with a hemoglobin of 80 per cent (Sahli), and a white-cell count of 6200, with a normal differential. The nonprotein nitrogen was 30 mg., the total protein 7.4 gm., the thymol turbidity 6 units, the gamma globulin 1.45 gm. per cent, and the total cholesterol 184 mg. per 100 ml., and the alkaline phosphatase 3 Bodansky units. Cephalin flocculation was negative. A bromsulfalein test revealed 2.8 per cent retention of the dye after 30 minutes. The patient was given a semisolid diet, sedation and antispasmodics but continued to vomit periodically, usually an hour or so after meals, stating that milk "curdled" in her stomach and caused the emesis. She remained weak and depressed. The abdomen was always scaphoid and nontender. Because of the persistent vomiting she was placed on a liquid diet, with antispasmodics by hypodermic injection. Only minimal improvement was noted on this regimen. Since the ward physicians had never witnessed any vomiting episodes, they thought that they were dealing with a reactive-depressive neurosis. On the 11th hospital day a barium-meal examination of the stomach disclosed a normal esophagus, slight irregularity of the cardia of the stomach, with admixture of retained food material, and an area of constriction in the antrum, with marked distortion of the mucosal pattern (Fig. 1). The 6-hour film revealed some distribution of the barium throughout the small bowel, most of the barium remaining in the stomach (Fig. 2). The diagnosis was corrosive pyloric stenosis.

The patient was transferred to a surgical service, where she continued to vomit intermittently. There was no free acid in the gastric juice even on histamine stimulation. On February 5, 1954, exploration under general anesthesia revealed a thickened stomach wall with stenosis of the distal 8 cm. of the pyloric antrum. The duodenal bulb was normal. A retrocolic gastrojejunostomy was performed with the posterior wall of the antrum proximal to the stenosis anastomosed to the jejunum. The postoperative course was uneventful, and she was discharged to the outpatient clinic on February 12.

When seen in March in the gastrointestinal outpatient clinic she felt fairly well and had gained 1.8 kg. (4 pounds). She stated that emesis was occasionally present after heavy foods. She still felt weak and was slightly depressed. Her relatives reported that she was drinking excessively.

CASE 2. R.D., a 49-year-old man, was transferred to Cook County Hospital as an emergency on January 4, 1955, from

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a private hospital, where lavage with baking soda and milk had been carried out after ingestion of 80 to 120 ml. of a soldering acid 3 hours previously. On admission, he was retching and moaning with pain and frequently vomited small amounts of white, curded material. Because he was confused and apprehensive an adequate history was obtained with difficulty. He stated that his wife had died recently and that, feeling depressed and lonely, he had swallowed the soldering acid in an attempt to end his own life.

FIGURE 1. *Constricting Deformity of the Antral Portion of the Stomach in Case 1 Six Weeks after Ingestion of an Acid-Containing Disinfectant.*

Physical examination revealed a well developed man complaining of abdominal pain. Small, white, macular areas were scattered over a hyperemic buccal mucosa, tongue, epiglottis and oropharynx. The heart and lungs were within normal limits. The abdomen was scaphoid. Epigastric tenderness was elicited on deep palpation. No rebound tenderness or palpable masses were noted. The bowel sounds were normal. Rectal examination revealed a foul-smelling, grayish-brown stool with a positive benzidine reaction.

The temperature was 99°F. by mouth, the pulse 100 and regular, and the respirations 10. The blood pressure was 140/80.

Laboratory studies revealed a blood nonprotein nitrogen of 38 mg. per 100 ml.; the sodium was 131 milliequiv., the chloride 91 milliequiv. and the potassium 4.3 milliequiv. per liter, and the carbon dioxide combining power 56 vol. per cent. The hemoglobin was 95 per cent (Sahli), and the white-cell count 19,800, with 81 per cent neutrophils, 12 per cent band forms, 6 per cent monocytes and 1 per cent lymphocytes.

The diagnosis was acid burns of the oropharynx, esophagus and upper gastrointestinal tract. Treatment consisted of demulcents, antibiotics and a liquid diet.

On the next day the patient felt much better and was in no distress. On the 3d hospital day he became dyspneic and complained of severe epigastric pain. He did not vomit [illegible] could swallow fluids. The abdomen was distended [illegible] rigid. The bowel sounds were faint and infrequent [illegible] marked tenderness, with rebound tenderness, was p[illegible] over the entire abdomen. He was given fluids intraven[illegible] and Wangensteen suction was started. A surgical co[illegible] tion was requested. The surgical consultant though[illegible] the patient had a ruptured viscus, and advised imm[illegible] exploratory laparotomy.

The patient was accordingly transferred to the s[illegible] service, and a laparotomy was performed under [illegible] anesthesia on January 6, 61 hours after the acid ing[illegible] 100 ml. of turbid fluid was encountered in the per[illegible] cavity. The distal third of the stomach was gray and [illegible] ic appearing. A 50 per cent gastric resection with a[illegible] colic gastroenterostomy was performed.

Pathological examination of the resected distal half [illegible] stomach and 1 cm. of the duodenum revealed an i[illegible] serosa with fibrinous adhesions. The wall was d[illegible] thickened to 5 mm. The mucosa was pinkish green [illegible]

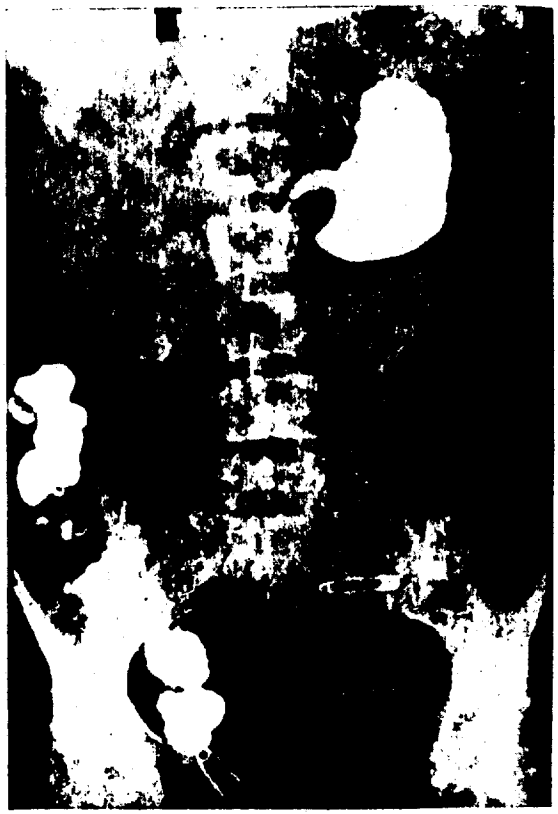

FIGURE 2. *Six-Hour Film in Case 1, Showing Persistence of the Constricting Deformity of the Antrum and M[illegible]ed Retention of Barium in the Stomach.*

A small amount of barium is noted in the distal [illegible]m and ascending colon.

numerous small erosions scattered over the surfa[illegible]. There was a cobblestone appearance to the intact mucosa (Fig. 3).

Microscopical examination of the stomach wall showed a severe acute gastritis with a marked inflammatory reaction in all layers of the wall and many zones of necrotic tissue.

The usual postoperative routine was followed. The patient was alert and co-operative for 3 hours after completion of the surgical procedures. On the 1st postoperative day he became anxious and tachypneic. Turbid, brown fluid with flecks of tissue was returned through the gastric suction tube. On the next day, the abdomen became distended, the temperature rose to 104°F. by mouth, and the dyspnea increased. Moist rales were heard over the right-lung field anteriorly and posteriorly. Because he was able to cough up

some purulent yellow sputum, bronchoscopic aspiration was not considered necessary. Penicillin and streptomycin were substituted in addition to oxytetracycline, which he had been receiving intravenously. In spite of carbon dioxide inhalation, alcohol and water sponges, he became cyanotic and died during the evening of the 2d postoperative day.

At autopsy the coroner's pathologist reported a gangrenous gastritis due to zinc chloride ingestion, focal fibrinous peritonitis and ileus, pneumonia of the right middle and upper lobes; acute esophagitis and tracheobronchitis; septic softening of the spleen; cloudy swelling of the myocardium and parenchymatous organs; and a recent gastric resection with acute duodenitis of the stump. The specimens of the stomach and duodenum revealed diffuse areas of gangrene involving almost the entire portion of the remaining stomach and the site of anastomosis with the jejunum (Fig. 4).

CASE 2. M.S., a seriously ill, 65-year-old man, was admitted to the medical service of Cook County Hospital on April 14, 1955. One hour previously he had attempted suicide by ingesting approximately 90 ml. of formaldehyde.

FIGURE 3. *Resected Specimen of the Stomach in Case 2, Showing Edema and the Marked Cobblestone Appearance of the Mucosa, with Numerous Small Erosions Scattered Throughout.*

Physical examination revealed a confused, elderly man in mild shock, complaining of severe generalized abdominal pains. The skin was cool and clammy. The abdomen was scaphoid; there was muscle guarding but no rebound tenderness. Bowel sounds were normal in character and frequency. No stool was obtained on rectal examination.

The pulse was 150 and regular but thready, and the respirations 36. The blood pressure was 100/70.

The diagnosis was corrosive gastritis due to formaldehyde.

Aspiration and washings of the stomach were initiated, with a return of approximately 100 ml. of foul-smelling, clear, yellow, benzidine-positive fluid. A urinary catheter was inserted, and 50 ml. of clear yellow urine with a specific gravity of 1.020 was obtained that was negative for albumin, sugar and acetone. Blood chemical examination revealed a sodium of 141 milliequiv., chloride of 96 milliequiv. and potassium of 4.9 milliequiv. per liter, with a carbon dioxide combining power of 48 vol. per cent. Examination of the blood showed a hemoglobin of 103 per cent Sahli and a white-cell count of 9100, with a normal differential. The blood Kahn test was negative, and an electrocardiogram was within normal limits.

FIGURE 4. *Autopsy Specimen in Case 2, Showing the Diffuse Areas of Gangrene Involving the Remaining Portion of the Stomach and the Adjacent Jejunum.*

Intravenous infusion of fluids and antibiotics was started. During the 1st 12 hours, hourly blood-pressure determinations recorded a progressive fall, necessitating intravenous administration of nor-epinephrine solution. On the 2d hospital day the patient was mentally alert and able to take fluids by mouth. The urinary output, however, was only 90 ml. for the first 24 hours, gradually increasing to 1200 ml. on the 4th day. The nonprotein nitrogen rose from 57 to 142 mg. per 100 ml. during this time, with a creatinine of 4 mg. per 100 ml. Temporary clinical improvement was noted on the 3d hospital day. The nor-epinephrine was discontinued and the patient was permitted to walk. Two days later acute diarrhea developed, with 15 to 18 bowel movements a day, consisting of small semiformed benzidine-positive stools. This was associated with generalized abdominal tenderness and distention. Oral feedings were stopped, and intravenous therapy was again started. On the following morning, rebound tenderness was elicited, with absent bowel sounds. An emergency film of the abdomen with the patient upright showed free air under the right hemidiaphragm. A surgical consultant advised immediate exploratory laparotomy. At operation a chemical fixation of the stomach with pneumatosis of the greater and lesser omentum and a sealed-over perforation of the lesser curvature in the pars pylorus was found. Immediate closure without further manipulation was considered the procedure of choice, and the operation was terminated as an exploratory one. The postoperative course was characterized by spiking

temperatures, mental lethargy and Kussmaul breathing although the abdomen remained soft and scaphoid and the bowel sounds again became active. Moist rales developed in both lung bases, and the patient died on the 3d postoperative day.

CASE 4. W.L., a 63-year-old man, was admitted to Cook County Hospital on September 8, 1955, about 8 hours after drinking an undetermined quantity of soldering fluid — probably in a suicidal attempt because of depression. Shortly after he drank the soldering fluid he was taken to a hospital

FIGURE 5. *Constricting, Smooth Deformity of the Lower Half of the Stomach in Case 4 Approximately Two Weeks after Ingestion of Muriatic Acid.*

in his vicinity for 1st aid. He was given 2 glasses of egg white and mineral oil and then sent to Cook County Hospital.

Physical examination showed a well developed and well nourished man lying quietly in bed who did not seem to be in severe distress. No signs of burns around the mouth were noted. The lips were red; the tongue was heavily coated with whitish material. There was no bleeding from the mouth, and there was little edema of the oropharynx. He could swallow water and speak with some difficulty. The neck was normal, as was the chest and its contents. The abdomen was flat and soft, without palpable masses. The bowel sounds were active. No abdominal tenderness was noted. There were no significant findings on examination of the back or the extremities.

The pulse was 84 and regular, and the blood pressure 170/80.

A diagnosis of corrosive gastritis was made, and the patient was put on a suitable regimen of liquid diet with demulcents, antispasmodics, sedatives and antibiotics.

Despite the instituted treatment whitish, tender plaques developed in the mouth, with swelling of the palate, and he started to vomit intermittently. He ran a slight temperature and began to lose weight. After dilatation of the lower esophagus with a No. 38 bougie, he seemed to be able to eat slightly better.

Urinalysis was negative. The hemoglobin was 105 per cent (Sahli), and the white-cell count 19,900 with 74 per cent neutrophils and 18 per cent band forms. The blood Wassermann test was negative. The nonprotein nitrogen was 59 mg., the creatinine 2.4 mg., and the total protein 7.5 gm. per 100 ml.; the sodium was 128 milliequiv., the potassium 4.5 milliequiv., and the chloride 93 milliequiv. per liter.

A gall-bladder film was normal, and barium-enema study was negative. X-ray examination of the stomach showed a smooth narrowing of the distal half of the stomach highly suggestive of cancer even though a stricture that had followed a corrosive substance was not ruled out (Fig. 5). A second gastrointestinal series 3 weeks later showed a more marked narrowing of the antrum, which now had a spindle-shaped appearance (Fig. 6).

Three weeks after admission gastroscopy, limited to the upper third of the stomach, revealed a boggy, hyperemic mucosa that bled easily. Supportive treatment was continued, and preparations made for operation.

At laparotomy on November 10 the stomach was found to be small, shrunken and adherent to the posterior wall. Because it was thought that any surgical procedure on the stomach at this time would be against good surgical judgment a jejunostomy was done, and the abdomen was closed. The patient made an uneventful recovery and was discharged 4 weeks later on jejunostomy feedings. He is being prepared for later gastric surgery (gastroenterostomy).

FIGURE 6. *Progressive Narrowing of the Lower Half of the Stomach, with Signs of Retention, Three Weeks after Figure 5 Was Taken.*

DISCUSSION

Corrosive gastritis results primarily from ingestion, either accidentally or with suicidal intent, of commercially available mineral acids such as hydrochloric, sulfuric, nitric and carbolic acids or corrosive alkalis. Alkalis exert their primary, destructive effect on the oral and esophageal mucosa, with only minimal involvement of the stomach, probably because of their neutralization by the highly acid gastric secretions. Contrariwise, acids, according to Boikan and Singer[1] and Meyer and Steigmann,[2] only sear the esophageal mucosa but flowing along the magenstrasse of the stomach, depending on the type and quantity of acid ingested, cause rapid coagulation necrosis of one or more layers of the stomach with the following se-

quelae: acute destruction of the gastric wall, with peritonitis; hemorrhage either immediately or in a few days to weeks; and destruction of the superficial layers, with edema and inflammation and loss of the gastric glands and intrinsic nerves and finally fibrosis with scarring.

The ingested agent flowing along the magenstrasse reaches the pyloric sphincter and antrum. Owing to the ensuing pylorospasm the acid remains at this site, where its most deleterious action takes place. More extensive involvement of the gastric mucosa will depend upon whether or not the corrosive poison followed the partaking of a meal. On the basis of pathological studies, Gradman, Gerber and Kaiser[3] state that the end result may be pyloric stenosis, antral stenosis, hour-glass deformity or linitis plastica.

The esophagus and stomach are severely injured by ingestion of a corrosive agent. If an alkaline corrosive (lye) is ingested, most of the damage occurs in the esophagus. If an acid corrosive is ingested the esophagus suffers less damage, most of the destruction occurring in the stomach. The damage to the stomach is definitely conditioned by the amount of gastric contents present in the stomach at the time the corrosive acid is ingested. From past experience with similar cases, we believe that it is possible to state from the x-ray appearance of a patient's stomach several weeks or months after ingestion of the acid whether or not the acid was taken on a full stomach. The patients who ingest the acid when their stomachs are full usually show a small pyloric narrowing, sometimes about 1 cm. wide, which could easily be diagnosed as annular carcinoma of the distal part of the antrum. They also present a huge, distended stomach. The patients who have ingested the acid on an empty stomach present a diffuse involvement of the lower half or two thirds of the stomach, only the upper, fundic portion remaining uninvolved, which may become quite distended compensatorily.

If the patient survives the first twenty-four hours and does not die from the immediate shock associated with the corrosive, widespread maceration and necrosis of the mucosa of the esophagus or stomach occur, as seen frequently by the coroner's pathologists. This may result in severe mediastinitis after lye poisoning, and gastritis with upper abdominal peritonitis after acid poisoning. During this stage (a week or longer) the patient is still in danger because the mediastinitis or gastritis and peritonitis may be so severe that the tissues cannot recover and the patient ultimately dies.

However, if the injury, as determined by the amount ingested and concentration of the corrosive substance, is not overwhelming, the patient usually responds to a careful supportive therapeutic regimen, which should consist of sedatives, antibiotics, blood transfusions, fluids, with attention to the electrolyte balance, antispasmodics and various demulcents given by mouth.

It is important to keep in mind not to use any large stomach tube for stomach lavage when the patient is first seen after the ingestion of a corrosive agent because such a tube may increase the damage to the gastric or esophageal wall. However, weak emetics may be used, or preferably only demulcents such as egg white, milk and olive oil. These dilute as much as possible any irritant that is still present. For some cases a specific antidote can be given -- for example, weak alkali for acids, lemon juice for alkali, plain salt for silver nitrate, and alcoholic beverages for carbolic acid.

During the acute stage when the patient presents the symptoms of peritonitis with tender, rigid, painful abdomen the dangerous impulse to perform an emergency laparotomy must be resisted. The experienced surgeon as a rule does not intervene during this stage because there is no actual peritonitis such as that seen after perforation of a hollow viscus. The rigid and painful abdomen is due to an irritation of the peritoneum by a necrotic organ without actual perforation (diffuse peritonitis). In this type of peritonitis, which is similar to that seen in severe cases of typhoid fever, regional ileitis or ulcerative colitis without actual perforation of the bowel, no surgery is indicated.

Occasionally, an erroneous diagnosis of peritonitis from a ruptured viscus is made because there is no history of intake of a corrosive substance. If the surgeon opens such an abdomen and finds a highly inflamed, almost gangrenous organ he should not start any surgical procedures but should only do a jejunostomy and close the abdomen. The jejunostomy may be useful to keep up the nutrition of the patient.

If the damage is not too severe so that the patient survives the first few days, nature as a rule will take care of the burned organ, by causing a slowly developing demarcation between the corroded and unaffected parts of the stomach. The patient may thus remain asymptomatic for weeks or even months. Then, depending on the pathologic changes that have occurred, he will present himself to the physician with one or more of the following symptoms: weight loss, anorexia, nausea, bloating, eructation, epigastric fullness and vomiting, either persistent or intermittent. Physical examination may reveal inanition, emaciation, dehydration, visible peristaltic waves in the upper abdomen or other signs of partial or complete pyloric obstruction, and laboratory examination may indicate severe electrolyte disturbance.

The course and appearance of symptoms described above are well illustrated by Cases 1 and 4, which also demonstrate the value of withholding operation and giving supportive treatment in these cases. The surgical intervention about eight and ten weeks after ingestion of the strong corrosive acid had much happier results for these patients than the laparotomy and subsequent gastric resection had during the acute stage in Case 2 and the laparotomy alone in Case 3.

It is obviously much easier for the surgeon to perform a gastroenterostomy on a stomach in which definite demarcation has taken place between the healthy and destroyed part of the stomach than to work on a stomach whose walls are macerated and necrotic. We therefore believe that a history of ingestion of a corrosive substance is a definite contraindication to an early laparotomy.

In most cases the various corrosive substances seem to have a definite predilection for damaging a specific organ. Thus, esophageal damage usually follows lye, and gastric damage acid ingestion. One finds, however, occasional reports of esophageal stricture in association with pyloric stenosis from acid ingestion[4] and of the coexistence of pyloric and esophageal lesions from lye.[5] One of us (F.S.) observed the presence of a slight esophageal stricture in a patient who had a severe pyloric stenosis after taking hydrochloric acid.

In the differential diagnosis of pyloric stenosis, especially in younger patients, one must consider the possibility of sequelae of corrosive gastritis. A diagnosis of carcinoma is often made[6,7] if an adequate history is not obtained. The history may not be obtained either because patients will not give it, if the acid ingestion has been in a suicidal attempt, or because the patient may not associate his present symptom with an occurrence several weeks or months previously. On the other hand, occasionally, the physician may erroneously regard the presenting symptoms described above as signs of a depressive neurosis because he fails to associate the previous suicidal attempt with the present condition.

Summary and Conclusion

The entity of corrosive (acid) gastritis, with its sequelae of pyloric stenosis, has received little attention in the American literature. It is not common knowledge that caustic alkalis exert their liquefaction necrosis primarily on the oral and esophageal mucosa, with little if any effect — in most cases — on the gastric mucosa because of the neutralizing action of the stomach secretions. Acids, in contrast, are notorious for their coagulation necrosis of the gastric mucosa with acute and chronic sequelae. The acute symptom may include hemorrhage, shock and "perforation" with peritonitis. The late sequelae may be classified according to the amount of destruction and the resultant scar formation: pyloric stenosis, central stenosis, hour-glass deformity or rarely total gastric scarring resulting in a linitis plastica. The obstructive symptoms can begin anywhere from a few weeks to years, with an average of four to six weeks, after the acute phase of corrosive poisoning has subsided. The treatment of the acute state is supportive — medical. The treatment of the sequelae is surgical — pyloroplasty or gastroenterostomy, depending on the pathology found at laparotomy. Subtotal gastric resection is rarely indicated.

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Suntzeff-'40
Bib No. 439

Amer. Jour. Cancer 39(1):56-60. 1940.

THE DEVELOPMENT OF SARCOMA IN MICE FOLLOWING LONG CONTINUED INJECTIONS OF A BUFFERED SOLUTION OF HYDROCHLORIC ACID [1]

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In former investigations the development of sarcoma was observed in several mice injected subcutaneously with various solutions of ovarian hormones, or with liver extract prepared for the treatment of pernicious anemia. As far as epithelial tissues are concerned, ovarian hormones induce a cancerous transformation only in the secondary sex organs, in which they cause rhythmic growth during the sexual cycle, or during pregnancy. This suggested that the factors causing the development of sarcoma might be less specific than those which induce the formation of carcinoma. In order to test this conclusion further, a number of mice were injected with solutions of substances which are not considered carcinogenic.

Fifty-five mice were injected subcutaneously for variable lengths of time. Table I shows the substances used for injection and their concentrations, the length of the period of injection, and the results obtained. The age of the mice given in the table is the age at examination at the end of the experiment. The large majority of the animals were males; only a few were females. The injections were given six times a week, except those of estrogen dissolved in oil. In addition to the experiments included in this table, nine mice belonging to strains A, C57, D and AKA were injected subcutaneously for a shorter time, namely for one month, six times a week, with 0.25 c.c. of a saturated solution of cane sugar. In most of these mice acute necrosis developed at the site of injection and the animals became cachectic; the experiment was therefore discontinued.

Of the various substances used for injection, only the solution of hydrochloric acid, kept at pH 5 by means of phthalate buffer, gave positive results; among 8 of the mice injected with a very dilute solution, 4 developed sarcoma at or near the site of injection. The findings in these mice were as follows:

(1) Male mouse, strain A, fourteen months old, weighing 28 gm., was injected with 0.25 c.c. of a HCl solution kept at pH 5 by means of phthalate buffer, six times weekly for ten and a half months. Two separate nodes of firm consistency were found attached to the muscle tissue in the dorsal region near the site of the injections. Microscopic examination showed a spindle-cell sarcoma, in which the cells were arranged in interlacing bundles. The tumor cells on the whole were small and of equal size, but some variations existed in this respect and there were cells and nuclei which were larger than the average. The cytoplasm of the sarcoma cells was often drawn out into longer fibers. The sarcoma penetrated into the surrounding dense hyaline tissue and, in other areas, into the dorsal musculature. The penetration took place in the direction of the longitudinal fibers of the muscle, and the

[1] These investigations were carried out with the aid of grants from the International Cancer Research Foundation and from the Jane Coffin Childs Memorial Fund for Medical Research.

TABLE I

(1) DISTILLED WATER, 0.5 c.c.: 7 mice, no sarcomas

1	male,	strain	A,	18.5	mos.	old,	injected	for	15.5	mos.
2	"	"	D,	17.5	"	"	"	"	15.5	"
1	"	"	D,	8	"	"	"	"	6	"
1	"	"	C57,	16	"	"	"	"	12	"
1	"	"	C57,	14	"	"	"	"	11	"
1	"	"	AKA,	17.5	"	"	"	"	16	"

(2) HYDROCHLORIC ACID, 0.25 c.c., buffered to pH 5 with 1.02 per cent acid potassium phthalate (KHC HO): 8 mice, 4 sarcomas at injection site

1	male,	strain	A,	14	mos.	old,	injected	for	10.5	mos.	Sarcoma
1	"	"	A,	16.5	"	"	"	"	13	"	Sarcoma
1	"	"	D,	17	"	"	"	"	15	"	Sarcoma
1	"	"	D,	18	"	"	"	"	16	"	No sarcoma
1	"	"	C57,	15	"	"	"	"	12.5	"	Sarcoma
1	"	"	C57,	18.5	"	"	"	"	16	"	No sarcoma
1	"	"	AKA,	13.5	"	"	"	"	11.5	"	No sarcoma
1	"	"	AKA,	17.5	"	"	"	"	15.5	"	No sarcoma

(3) TEN PER CENT SODIUM SULFATE, 0.25 c.c. daily: 9 mice were injected

1	male,	strain	A
2	females,	"	A
2	males,	"	D
2	"	"	C57
2	"	"	AKA

All of these mice, except one male, were injected for about twelve months and all died without developing tumors. One male was injected for 15.5 months, and was 18.5 months old when killed.

(4) GELATIN (KNOX), 1 per cent in 0.9 per cent NaCl solution, 0.25 c.c.: 4 mice, no sarcomas

1	male,	strain	A,	18.5	mos.	old,	injected	for	15.5	mos.
1	female,	"	A,	11	"	"	"	"	8.5	"
1	male,	"	C57,	12.5	"	"	"	"	10	"
1	"	"	AKA,	17.5	"	"	"	"	16	"

(5) TWENTY-FIVE PER CENT GLYCERIN (by volume), 0.25 c.c.: 7 mice, no sarcomas

1	male,	strain	A,	14	mos.	old,	injected	for	11	mos.
1	"	"	D,	11	"	"	"	"	9	"
1	"	"	D,	18.5	"	"	"	"	16	"
1	"	"	C57,	18.5	"	"	"	"	16	"
1	"	"	C57,	18.5	"	"	"	"	16	"
1	"	"	AKA,	10	"	"	"	"	8	"
1	"	"	AKA,	17.5	"	"	"	"	15	"

(6) PROGYNON B (SCHERING) dissolved in 0.25 c.c. water, 20 rat units: 5 mice, no sarcomas

1	male,	strain	D,	11	mos.	old,	injected	for	8.5	mos.
1	"	"	D,	13.5	"	"	"	"	11	"
1	"	"	C57,	19	"	"	"	"	16.5	"
1	"	"	C57,	12.5	"	"	"	"	10	"
1	"	"	AKA,	18	"	"	"	"	16.5	"

(7) PROGYNON B (SCHERING) dissolved in oil, 100 rat units: 6 mice injected once a week subcutaneously, no sarcomas

1	male,	strain	A,	8.5	mos.	old,	injected	for	5.5	mos.
1	female,	"	A,	11	"	"	"	"	8.5	"
1	"	"	A,	19	"	"	"	"	16.5	"
1	male,	"	D,	8	"	"	"	"	5.5	"
1	"	"	C57,	9	"	"	"	"	6.5	"
1	"	"	AKA,	9	"	"	"	"	7.5	"

nuclei of the sarcoma cells became drawn out during this process. In various places isolated fibers were still visible between the strands of tumor cells. Numerous mitoses were seen in the sarcoma cells in some areas, but in others were less abundant.

(2) Male mouse, strain A, sixteen and a half months old, weighing 25 gm., was likewise injected with 0.25 c.c. of a HCl solution kept at pH 5 by means of phthalate buffer, six times a week for a period of thirteen months. A tumor somewhat larger than a hazelnut

was found at or near the injection site. Microscopic examination showed it to be a sarcoma consisting of relatively small spindle cells; giant cells were lacking. Usually the cells and nuclei were drawn out in the direction of the interlacing strands. A considerable number of mitoses was found. In addition to the sarcoma cells there were present mononuclear cells with markedly eosinophilic cytoplasm. These cells are probably wandering eosinophilic cells which are frequently found in various organs of the mouse. In addition many lymphocytes were seen in the sarcoma.

(3) Male mouse, strain D, seventeen months old, weighing 23 gm., was injected with 0.25 c.c. of the same kind of HCl solution, six times a week for a period of fifteen months. A solid tumor about the size of a hazelnut had developed on the back near the rump, at or near the place where the injections had been made. Microscopic examination showed a spindle-cell sarcoma with many mitoses in the spindle cells. On the whole the tumor cells were rather small; but in places they were larger and long drawn out, especially in the peripheral areas, where they penetrated into the surrounding muscle and fibrous tissue; their movement, as a rule, was in the direction of the fibers of these structures. The cells were likewise of larger size where they penetrated into loose connective tissue, the interstices of which were apparently filled with a coagulated plasma.

(4) Male mouse, strain C57, fifteen months old, weighing 30 gm., was likewise injected with 0.25 c.c. of the same kind of HCl solution, six times a week for a period of twelve and a half months. As in the cases just cited, a good-sized solid tumor developed at the site of the injections. Microscopically the growth was found to be a spindle-cell sarcoma. Numerous mitoses were observed in the spindle cells, which, on the whole, were large and included large nuclei, in many of which a single large nucleolus was seen. There were also binucleated cells and cells with a chain of nuclei. Giant cells were noted, being especially frequent near areas of necrosis. Some large mitoses were visible. The tumor penetrated into the muscle tissue, which was destroyed by the invading cells. Elsewhere a fibrous capsule was present.

The starting point for these experiments was the observation that in several animals we had noticed the development of sarcoma at or near the place where long-continued injections of estrogenic or luteal substances had been made, and in one instance we had found a sarcoma after injection of a liver extract used in the treatment of pernicious anemia. Only once did the sarcoma develop in the vaginal-cervical region, at a point far removed from the injection site. We concluded that these sarcomata originated under conditions less specific than those which as a rule induce the formation of carcinoma.

In order to test further this relative lack of specificity of the stimuli which produce sarcoma in mice, we injected a number of relatively simple substances, such as distilled water, solutions of HCl buffered at pH 5, glycerin, gelatin, and sodium sulfate, as well as solutions of estrogen dissolved in water or in oil. These injections extended in the large majority of cases over periods varying between eleven and sixteen months. While the number of mice treated in the various experiments is limited, we may conclude that of the substances which we used, HCl solutions buffered with phthalate are the only ones able to induce sarcoma formation. The possibility is not excluded, however, that if a large number of animals had been used, sarcoma might have developed following some of the other injections. While these negative experiments can, therefore, be recorded only with this reservation, the results obtained with the injection of the buffered solution of HCl are significant.

Among 8 mice thus injected over periods varying between ten and a half and fifteen months, 4 developed spindle-cell sarcoma in the subcutaneous tissue at or near the injection site. In 4 other mice injected with the same substance

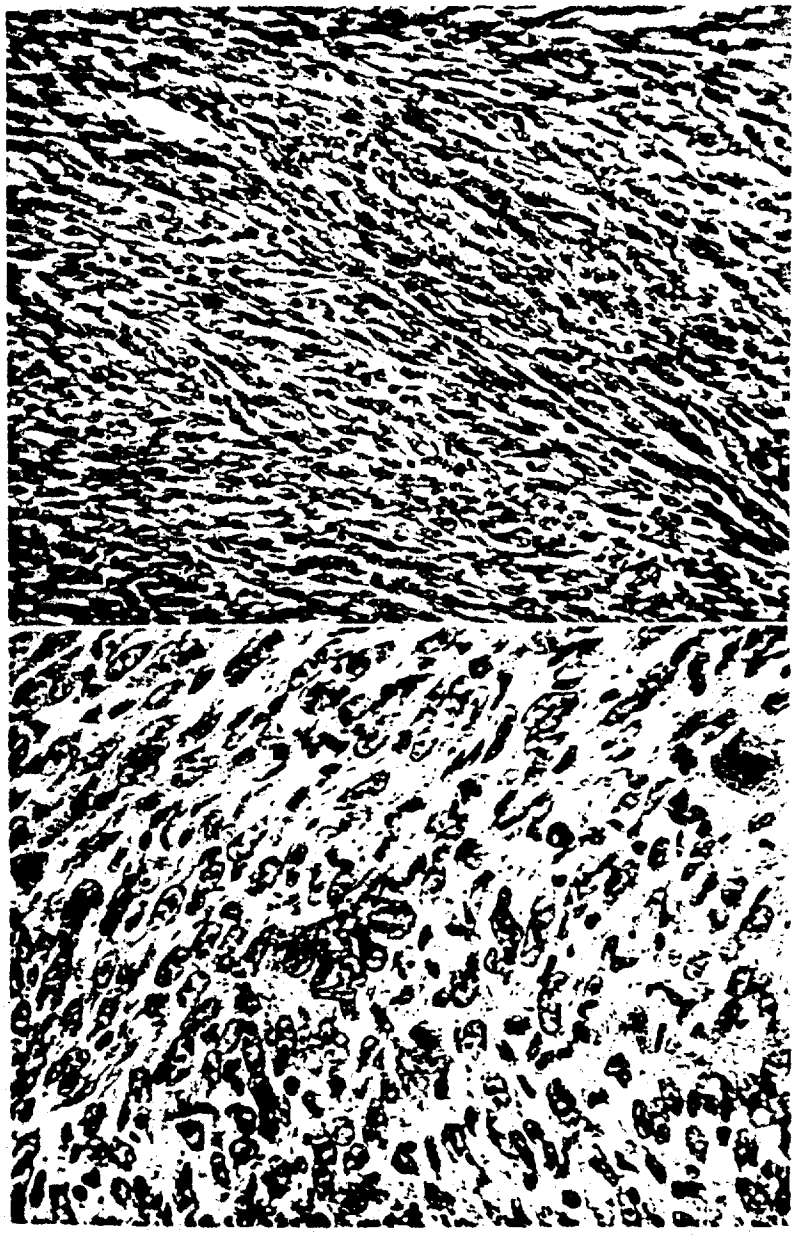

FIG. 1. SARCOMA IN A MALE STRAIN D MOUSE WHICH HAD BEEN INJECTED WITH 0.25 C.C. OF A HCL SOLUTION KEPT AT pH 5 WITH PHTHALATE BUFFER FOR FIFTEEN MONTHS: STRANDS OF SPINDLE CELLS: MITOSIS VISIBLE IN CENTER

FIG. 2. SARCOMA IN A MALE STRAIN C57 MOUSE WHICH HAD RECEIVED SIMILAR INJECTIONS FOR TWELVE AND A HALF MONTHS: GIANT CELLS AND MITOSES

We are indebted to Mr. S. J. Hayward for the photomicrographs.

for periods varying between eleven and a half and sixteen months, sarcoma did not arise. The mice in which positive results were obtained belonged to strains A, D and C57, which vary greatly in the tendency to the development of spontaneous mammary carcinoma. The genetic constitution of the strain

which predisposes to the development of sarcoma from the subcutaneous connective tissue, or from striated muscle tissue, is therefore different from the genetic factors which are involved in the origin of mammary gland carcinoma, a conclusion which agrees with the findings of various investigators, tending to prove that hereditary factors are specific for each particular type of tumor.

As to the factor directly responsible for the development of sarcoma in these cases, it is not certain whether it was the acid as such or the 1.02 per cent solution of acid potassium phthalate which served as buffer. There exists the possibility that impurities present in the phthalate solution were the real cause of the sarcomatous transformation. Considering the fact, however, that the solution injected daily contained 2.5 mg. of phthalate, the amount of admixture would in all probability have been too small to be effective.

As stated above, the experiments in which 0.25 c.c. of a saturated cane sugar solution was injected into mice six times a week for one month had to be abandoned on account of necrosis at the point of injection. Quite recently, however, we have learned that Takizawa, as well as Nonaka, observed the development of sarcoma at or near the site of injections after long-continued injection of 20 per cent or 25 per cent solutions of glucose into the subcutaneous tissue in rats and mice. The shortest time after which the sarcoma arose was eight months. In one mouse injected with fructose a fibrosarcoma developed. Simultaneous feeding with orthoamidoazotoluol seemed to accelerate the effect of the sugar solutions. Of Nonaka's rats, 3 out of 4 surviving longer than 400 days developed either spindle-cell sarcoma or fibrosarcoma.

Our experiments, as well as those reported by the Japanese investigators, leave little doubt that chemically relatively simple substances, other than the well known carcinogenic compounds, are able to produce sarcoma after long-continued injection. There are indications, also, that the development of sarcoma as well as of carcinoma takes place by intermediate steps, and that the formation of a fibrosarcoma may represent one of the transitional stages.

Summary

In continuation of previous experiments which indicated that the range of factors which may induce the production of sarcoma is greater than the range of factors which induce the production of carcinoma, and that the former conditions are correspondingly less specific than the latter, it was found that injection of 0.5 per cent HCl buffered with phthalate can produce sarcoma in the subcutaneous tissue of mice if the injections are continued over a relatively long period of time. Other solutions tested, as distilled water, sodium sulfate, glycerin and gelatin, have not, so far, led to positive results. Sarcoma could be produced in strains in which mammary gland carcinoma arose spontaneously only very rarely, as well as in strains in which mammary gland carcinoma developed readily under the influence of ovarian hormones.

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Telfer-'39
Bib No. 452

Glasgow Medical Journal

New (7th) Series June, 1939 Vol. XIII No. VI

ORIGINAL ARTICLES

THE INFLUENCE OF ACID-FEEDING ON THE UTILIZATION OF THE MINERAL ELEMENTS.

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In a contribution to the study of mineral metabolism in infancy published in this *Journal* (1930), reference was made to the treatment of tetany by acid-feeding. The method, which is described later, was based on the results obtained in the following experiment:—

A child of six years was maintained on an accurately measured quantity of fresh cow's milk for six days, during which the urine and fæces were collected separately and quantitatively (Period I.). Hydrochloric acid was added to the milk till the acid concentration was approximately $\frac{N}{20}$, and after a pre-period of three days to establish metabolic equilibrium, the excreta were collected as before over two consecutive periods of six and seven days (Periods II. and III.). The acid was then withdrawn, and at the close of a pre-period of three days on cow's milk only, the excreta were collected during a final period of seven days (Period IV.). The urine corresponding to each period was diluted to a known volume, and the fæces dried and weighed. A sample of the milk was taken for the estimation of calcium magnesium and phosphorus, from which the mineral intake was calculated. The distribution of the mineral elements in the excreta and the total mineral output were determined in the usual manner by analysis of the urine and fæcal solids, the difference between intake and output expressing the retention of each element. The results are recorded in the following Table:—

TABLE I.—V.P. ÆT. 6. 11·56 KILOS. DIET: 1,800 C.C. COW'S MILK PER DIEM.

	Period I. Milk only, 6 days.			Period II. Acid feeding, 6 days.			Period III. Acid feeding, 7 days.			Period IV. Milk only, 7 days.		
RETENTION OF MINERAL ELEMENTS (grams).												
	CaO	MgO	P_2O_5	CaO	MgO	P_2O_5	CaO	MgO	P_2O_5	CaO	MgO	P_2O_5
Intake - -	17·28	2·46	23·76	17·28	2·46	23·76	20·16	2·89	27·72	20·16	2·89	27·72
Output - -	7·05	1·61	11·34	9·13	1·82	13·29	10·12	2·39	16·15	11·83	2·29	16·96
	10·23	0·85	12·42	8·15	0·64	10·47	10·04	0·50	11·57	8·33	0·60	10·76
Retention per kilo. per day	0·15	0·01	0·18	0·12	0·009	0·15	0·13	0·006	0·15	0·11	0·007	0·14
DISTRIBUTION OF MINERAL ELEMENTS IN THE EXCRETA (grams).												
	CaO	MgO	P_2O_5	CaO	MgO	P_2O_5	CaO	MgO	P_2O_5	CaO	MgO	P_2O_5
Urine - - -	0·12	0·36	7·14	0·90	0·67	9·54	1·19	0·85	11·41	0·28	0·39	8·4
Fæces - -	6·93	1·25	4·20	8·23	1·15	3·75	8·93	1·54	4·74	11·55	1·90	8·56
	7·05	1·61	11·34	9·13	1·82	13·29	10·12	2·39	16·15	11·83	2·29	16·96
ANALYSIS OF FÆCAL SOLIDS (per cent).												
Mineral Matter	24·44			27·1			30·56			34·18		
Lime (CaO)	10·35			14·45			15·35			13·50		
Magnesia (MgO)	1·87			2·03			2·66			2·23		
Phosphoric Acid (P_2O_5)	6·27			6·59			8·15			10·00		
Neutral Fat	9·53			5·22			5·73			4·86		
Free Fatty Acids	4·52			3·28			1·37			1·64		
Combined Fatty Acids	26·90			26·80			25·60			27·40		
Total Fat	40·95			35·30			32·70			33·90		
$\frac{CaO}{P_2O_5}$ (fæces)	$\frac{1·6}{1}$			$\frac{2·2}{1}$			$\frac{1·9}{1}$			$\frac{1·3}{1}$		
$\frac{\text{Urinary Phosphorus}}{\text{Fæcal Phosphorus}}$	$\frac{1·7}{1}$			$\frac{2·5}{1}$			$\frac{2·4}{1}$			$\frac{0·9}{1}$		

Fig. 1. shows the changes in the renal output of the mineral elements following acid ingestion. (Per cent. of total output).

RESULTS.

The data show that the distribution of the mineral elements in the excreta is widely altered by the ingestion of hydrochloric acid. In the first Period, 1·7 per cent of the unretained lime was excreted in the urine, 22·4 per cent of the magnesia and 62·9 per cent of the phosphoric acid (*v.* chart). These values were increased to 9·8 per cent CaO, 36·8 per cent MgO, and 71·7 per cent P_2O_5 in Period II., and maintained at this high level in

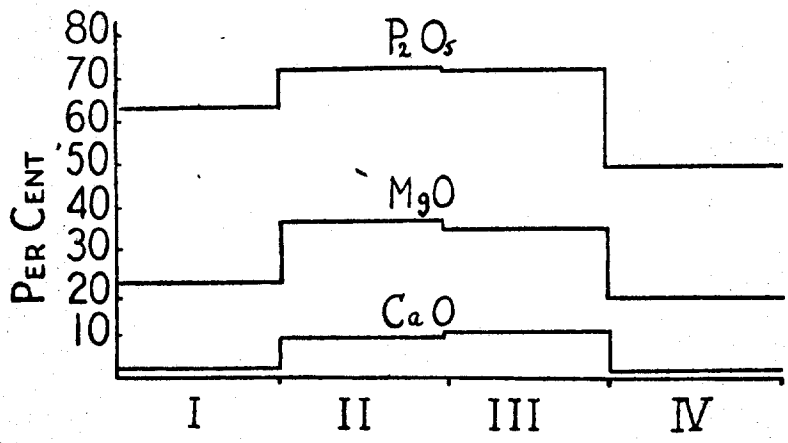

FIG. 1.

Period III. During the latter period nearly ten times as much lime was eliminated in the urine as in Period I., and the renal output of magnesia was more than doubled. On withdrawal of the acid (Period IV.), an abrupt fall in the urinary excretion of all three elements took place, the lime value being reduced to 2·3 per cent, the magnesia to 17·0 per cent and the phosphoric acid to 49·6 per cent.

In Period I. the percentage of neutral fat (9·53) and of total fat (40·95) were slightly above the normal, and the concentration of fatty acids in combination with the basic elements was relatively high (26·9 per cent). As shown in earlier work on mineral excretion (1921) a high percentage of combined fatty acids in the fæcal solids is associated with a large renal output

of phosphoric acid. Less phosphorus is restricted to the intestine in the form of phosphates owing to the increased combination of the basic elements with fatty acids as insoluble soaps. In spite of the existence of this mode of excretion in the initial period, the administration of the acid was followed by a considerable rise in the renal output of phosphoric acid which coincided with a fall in fæcal output (Periods II. and III.). These changes are illustrated by the increase in the ratio of lime to phosphoric acid in the fæces and of urinary to fæcal phorphorus. When the acid was discontinued (Period IV.), both ratios were markedly reduced, the renal output of phosphoric acid being lowered and the fæcal output increased.

The retention values were slightly diminished by the acid ingestion and the high degree of retention found in the initial period was not immediately regained when the acid was withdrawn (Period IV.). In the two intermediate Periods the quantities retained and the renal output were greater than in Period IV.: but the rise in the renal output of magnesia and phosphoric acid, following the addition of acid to the milk (Period II.), barely exceeded the fall in retention.

Discussion.

In a series of experiments carried out in 1880, Schetelig found that in man the administration of hydrochloric acid was followed by an increased urinary excretion of calcium and phosphorus. Later investigations by Rüdel (1893), Folin and Schaffer (1902), Adler (1908) and others fully confirmed this observation (1914). The present experiment shows that magnesium is also involved and that the rise in renal output of all three elements is associated with a decrease in the amount of phosphate eliminated in the fæces and a fall in retention. Two effects are indicated by these changes—an increased absorption of the mineral elements and a reduction in the quantities normally fixed by tissues in the process of growth.

In almost all foodstuffs the mineral elements exist as insoluble compounds and the first step in assimilation is their conversion into soluble form to permit of absorption. Solution is effected by the free acid of the gastric juice. The intestinal secretions, however, are alkaline and the basic elements, which exist partly as phosphates, tend to undergo precipitation as soon as

the chyme in which they are held in solution passes into the duodenum. The maintenance of an acid reaction in the small intestine is clearly an essential condition of absorption.

Experiment on animals has shown that the acidity due to the gastric secretion is rapidly reduced in the duodenum and lessens continuously to about the middle of the ileum where the reaction is definitely alkaline. From these and other observations, it has been inferred that the area over which free mineral absorption takes place is restricted to the upper part of the gastro-intestinal tract, and that the fall in acidity of the intestinal contents imposes a limit on the absorption of the basic elements (1923).

An increased absorption of calcium, magnesium, and also of iron might therefore be expected from the ingestion of hydrochloric acid, since the excess of acid would tend to enlarge the area over which the basic elements could exist in solution in presence of phosphoric acid.

The data are in agreement with this conclusion. A diet consisting exclusively of cow's milk ($CaO = 0{\cdot}16$, $MgO = 0{\cdot}02$ and $P_2O_5 = 0{\cdot}21$ per cent) and of adequate calorific value provides a large excess of calcium magnesium and phosphorus above requirements. Although a considerable proportion of the intake of each element escapes absorption, the amount absorbed is normally greater than the quantity retained and fixed by the growing tissues. In healthy children 50 to 60 per cent of the excess of phosphorus is excreted in the urine. The elimination of this element mainly as an acid radical entails the removal of an equivalent amount of basic substances; but in comparison with other available bases the alkaline earths are feebly soluble in combination with phosphoric acid, and as a rule less than 20 per cent of the excess of magnesium and only about 2 per cent of the calcium are excreted in the urine. When an excess of calcium is administered intravenously it is partly excreted into the intestine. The renal output of the alkaline earths nevertheless increases appreciably with the quantities absorbed and with the amount of phosphoric acid transported through the kidney. If a higher degree of absorption of the mineral intake were induced by acid administration it would therefore be followed by a larger urinary excretion of all three elements; but the rise in the renal output of phosphoric acid and the fall

in fæcal output would be much greater than the corresponding changes in the distribution of lime and magnesia.

While this effect is indicated by the increase in the ratio of urinary to fæcal phosphorus, and of lime to phosphoric acid in the fæcal solids (Periods II. and III.), the changes in the mode of excretion of the mineral elements cannot be attributed solely to the absorption of a larger proportion of the mineral intake. The retention values are definitely lowered by acid ingestion, so that the very considerable rise in the renal output of the two basic elements with the phosphoric acid is dependent not only on an increase in absorption, but on a reduction in the quantities utilized in growth.

Previous studies in mineral metabolism have shown (1922) that lime and phosphoric acid are retained in approximately equal quantities, the values in early childhood ranging from 0·06-0·12 gram per kilo of body weight per day. The retention of magnesia is about one tenth of the lime. Almost the whole of the lime and over 80 per cent of the phosphoric acid are deposited in the skeleton and the proportion in which they are fixed is very nearly constant (CaO 1·0 P_2O_5 0·8). A fall in retention from high to lower positive values consequently implies a lessened deposition of mineral matter in skeletal growth; and if the quantities absorbed are not diminished this change must necessarily be followed by the elimination of a larger excess of all the bone-forming elements, the calcium partly by re-excretion into the gut. Otherwise the concentration of each element in the blood, or in the soft tissues would increase progressively. As shown later in the treatment of tetany by acid feeding, a considerable increase in the calcium content of the blood does occur when the level is abnormally low.

The fall in retention is of interest since it would appear to indicate that the process of calcification is adversely effected in circumstances tending to produce an acidosis. When hydrochloric acid is ingested, an excess of acid or of acid products is absorbed. Studies in acid-base equilibrium (1931) have shown that neutralization is immediately effected by buffer salts and mainly by the alkali bicarbonate of the plasma—$HA + BHCO_3 = BA + H_2CO_3$. The alkali reserve is therefore diminished and the ratio $H_2CO_3 : BHCO_3$ tends to increase. Acid-base equilibrium is restored through the removal of an

excess of CO_2 by the lungs following an acceleration of the respiratory rate, the H_2CO_3 concentration of the blood being lowered below the level at which it existed prior to acid ingestion.

A slight fall in the pH of the serum occurs, since the concentration of H_2CO_3 is lowered less rapidly than that of $BHCO_3$.

An excess of acid is gradually eliminated by the kidney in the form of ammonium salts, the withdrawal of acid radicals in combination with ammonia liberating a corresponding amount of base which is retained—$NH_4\ HCO_3 + BA = NH_4\ A + BHCO_3$. The loss of base is also restricted and the alkali reserve maintained by the excretion of a larger excess of acid as acid phosphates (BH_2PO_4). In the blood the alkali salts of phosphoric acid are feebly concentrated and of small importance as buffers in comparison with the bicarbonate, proteins, and organic phosphates. As shown by L. J. Henderson, only 20 per cent of the inorganic phosphate exists as acid salts, the ratio $B_2HPO_4 : BH_2PO_4$ at pH 7·4 being nearly 4 to 1, whereas in urine of average acidity (pH 6·0) about 80 per cent of the phosphorus is excreted in this form. The elimination of a much larger proportion of acid than of basic salts must therefore tend to increase the alkalinity of the blood. The process may be one of selective excretion, the basic salts being retained, or the latter may in part be transformed into acid salts with the retention of a corresponding amount of base, $B_2HPO_4 + H_2CO_3 = BH_2PO_4 + BHCO_3$. This mechanism is apparently accelerated in response to a diminution of the alkali reserve. After the ingestion of a mineral acid, almost the whole of the urinary phosphorus is excreted in the form of acid salts, and a "phosphaturia" is induced, the total renal output being increased. An excess of water and neutral salts is also eliminated by the kidney which may lead to dehydration and possibly to some loss in vital activity of the tissues.

Although the latter effect is suggested by the persistence of the diminished retention values of lime and phosphoric acid after the administration of hydrochloric acid had been discontinued (Period IV.), the fall in retention may be due in part at least to the disturbance of acid-base equilibrium produced by the absorption of an excess of acid. From the experimental

studies *in vitro* of Holt, La Mer and Chown (1925) it would appear that even a very slight fall in the pH of the tissue fluids may lessen appreciably the degree of supersaturation of the bone-forming elements, and tend to lower the rate of calcification. The increased expenditure of phosphoric acid in the removal of an excess of acid products may also be an adverse factor. The latter possibilities are considered more fully in a later part of this work.

In relation to the "phosphaturia of acidosis," it is evident that under the conditions of the present experiment the large renal output of phosphoric acid is primarily dependent on an increased absorption of phosphorus from the alimentary tract and secondarily on a reduction in the quantity retained. As shown by the *positive* retention values, and the wide alteration in the ratio of urinary to faecal phosphorus during the two intermediate periods, the excess of phosphorus eliminated by the kidney is derived from the intake, but it includes a small proportion of phosphorus which under normal conditions would be utilized in growth, probably with lime in the process of calcification. It may be of interest to recall that an excessive renal output of phosphoric acid and of lime and magnesia is a characteristic feature of the mode of excretion in jaundice (1921). In this condition the alkalinity of the intestinal contents is probably diminished by the presence of a large excess of free fatty acids and the exclusion of bile from the gut.

The Treatment of Post-Operative Tetany.

As indicated by the results of the present experiment, the ingestion of hydrochloric acid is followed by an increased absorption of all the components of the mineral intake and the absorption of an excess of acid or acid products which must tend to increase the calcium content of the blood. Earlier work on mineral metabolism (1920) has also shown that the amount of calcium absorbed increases with intake over a wide range. A more pronounced effect on the calcium content of the blood might therefore be expected if hydrochloric acid were admininistered with an excess of a calcium salt, and on the suggestion of the writer this procedure was adopted in the treatment of a number of cases of tetany resulting from operations on the thyroid gland, the removal of parathyroid tumours, and other causes.

The Influence of Acid-Feeding

In one of these cases, that of a woman of 37 years, there was reason to believe that the parathyroid glands had been completely extirpated. She was admitted to the surgical wards of the Western Infirmary, on 12th November, 1929, with a large cystic swelling on the left side of the neck, which interfered with breathing and swallowing. A similar swelling had been removed from the right side of the neck thirteen years previously. On 29th November, an operation was performed in which numerous cysts where shelled out from the thyroid gland. Severe tetany developed on the following morning, when examination showed that the calcium content of the blood had been reduced to about half the normal value. The symptoms were relieved by the administration of calcium chloride intravenously, and later by the injection of parathormone (Collip) 10 units, t.i.d. The calcium content of the blood remained abnormally low, however, and at the end of five days when the injections of parathormone were discontinued, the signs of tetany returned. Acid feeding was then adopted and the calcium intake greatly increased. Two litres of milk with approximately 100 cc. $\frac{N}{1}$ HCl and 120 grains of $CaCl_2$ were administered daily with immediate improvement in the condition of the patient. The changes produced are shown in the following table, which includes the data of two metabolic studies, one before (Period I.), the other three days after (Period II.), the commencement of acid feeding :—

TABLE II.

	Period I. (Tetany.)			Period II. (After acid ingestion.)		
	CaO.	MgO.	P_2O_5	CaO.	MgO.	P_2O_5
Retention (grams per day),	1·79	0·22	1·13	0·71	0·04	0·225
Renal Output (Grams 4 days),	0·21	0·37	9·22	1·12	0·72	11·79
Urinary volume (4 days),	5,300 cc.			6,630 cc.		
Titratable acidity (per 100 cc.),	29·2 cc. $\frac{N}{10}$ NaOH.			52·0 cc. $\frac{N}{10}$ NaOH.		
Ammonia, - - -	0·06 gr NH_3 per cent.			0·14 gr NH_3 per cent.		
CO_2 per 100 cc. of plasma,	65·0 cc.			58·0 cc.		
Calcium content of Serum,	0·006 gr Ca. per cent.			0·009 gr. Ca. per cent.		
Magnesium ,, ,, ,,	0·0014 gr Mg. ,, ,,			0·0018 gr Mg. ,, ,,		
Inorganic phosphorus ,,	0·005 gr P. ,, ,,			0·005 gr P. ,, ,,		

In the period of tetany, the magnesium as well as the calcium content of the serum was much below the normal level (0·002 gr Mg. per cent, 0·01 gr. Ca per cent), and the renal output of both elements very small. The urinary volume, the acidity of the urine, and the alkali reserve, as measured by the CO_2 combining power of the plasma, were within normal limits though somewhat high values were found for the retention of lime and magnesia. In healthy adults the total output of the bone-forming elements is almost equivalent to the intake.

The ingestion of hydrochloric acid (Period II.) was followed by a pronounced rise in the calcium and the magnesium content of the serum to which the relief from tetany may be attributed. The retention values of the mineral elements were diminished, though still positive and the renal output was greatly increased. An excess of acid was eliminated by the kidney as shown by the increase in urinary volume, the titratable acidity of the urine, and the renal output of

ammonia. The inorganic phosphate of the blood, which in the period of tetany was above the normal level (0·003 gr P per cent) in correlation with the low calcium content, remained unchanged, but the alkali reserve was lowered, presumably through the absorption of an excess of acid or of acid products.

Some weeks later anæmia began to develop and the feet and legs became œdematous. Acid administration was temporarily discontinued and the very low intake of iron afforded by the milk diet was increased. The œdema disappeared and recovery from the anæmia became progressive, but in spite of the large intake of $CaCl_2$ the calcium content of the serum fell to 0·006 gr Ca per cent, and on 3rd April, 1930, tetanic convulsions occurred. Acid feeding was resumed as before and the calcium content of the blood rose to 0·008 gr. Ca per cent, at which level it was maintained without recurrence of tetany or symptoms of acid intoxication.

The patient was discharged from hospital on 14th June, 1930, and from this date onwards biochemical examinations were made at intervals. No appreciable changes in the blood were found on 15th December, 1930, when the CO_2 of the plasma was 48·4 cc., the calcium content of the serum 0·009 gr Ca and the inorganic phosphorus 0·004 gr P per cent.

Three months later (14th March, 1931) acid feeding and the administration of $CaCl_2$ were discontinued. Severe tetany developed, the serum calcium falling to 0·0057 gr Ca and the phosphorus increasing to 0·006 gr P per cent. Again it was found that the ingestion of a large excess of $CaCl_2$ alone had practically no effect on the calcium content of the serum and that the administration of hydrochloric acid was still necessary to prevent the onset of tetany. After a few days in Hospital for observation the patient was sent home (14th May, 1931) with instructions to continue taking the $CaCl_2$, iron, and thyroid daily, and the acid in milk once a week. A relapse occurred on 4th October, 1931, when she was readmitted in tetany, the CO_2 of the plasma being 67·2 cc., plasma chloride 0·55 gr NaCl, serum calcium 0·006 gr Ca, and the inorganic phosphorus 0·005 gr P per cent. A gradual reduction in the intake of acid and of $CaCl_2$ was nevertheless effected without recurrence of tetany, and on 15th June, 1932, the only symptom of the illness was a slight stiffness of the arms. (CO_2 of plasma 54·0 cc., plasma chloride 0·54, Calcium of serum 0·0086, and inorganic phosphorus 0·005 per cent). No acid had been ingested during the previous three months.

On 12th March, 1936, she was admitted to hospital with signs of latent tetany. A slight reduction in the serum calcium to 0·007 gr Ca per cent had taken place and an increase in the inorganic phosphorus to 0·006 gr P per cent. No acid was given, but the mineral intake was raised by the addition of a liberal quantity of milk to the diet and cod-liver oil was administered. At the end of a fortnight with rest in bed, all symptoms had disappeared. (CO_2 of plasma 62.4 cc., plasma chloride 0·57 gr NaCl, serum calcium 0·008 gr Ca and inorganic phosphorus 0·0058 gr P per cent). No significant alteration in any of these values was found at the last examination made on 17th February, 1939.

Similar results were obtained in the case of a young woman admitted to Dr. Gracie's wards on 29th June, 1935, suffering from tetany, which had developed two months after a partial thyroidectomy for exophthalmic goitre. The symptoms were immediately relieved by the administration of 150-200 cc. $\frac{N}{10}$

HCl in milk thrice daily. Acid feeding was continued for several weeks, during which the calcium content of the serum was maintained at a relatively high level. No signs of acid intoxication were observed and she was discharged from hospital apparently well on 26th August, 1935. The changes produced by the acid ingestion are shown in Table 3.

TABLE III.

	Period I. (Tetany.)			Period II. (After acid ingestion.)		
	CaO.	MgO.	P_2O_5	CaO.	MgO.	P_2O_5
Renal Output (Grams 3 days),	0·109	0·21	2·13	0·38	0·33	4·08
CO_2 per 100 cc. of plasma,	62·0 cc.			53·0 cc.		
Chloride of plasma (NaCl),	0·55 gr per cent.			0·55 gr per cent.		
Calcium content of Serum,	0·0056 gr Ca. per cent.			0·0083 gr Ca. per cent		
Inorganic phosphorus ,, ,,	0·005 gr P. per cent.			0·005 gr P per cent.		

As in the first case, the plasma chloride was below, and the inorganic phosphorus above the normal concentration during the period of tetany and these values were unchanged by the administration of the acid, which was followed by a considerable increase in the calcium content of the serum, and in the renal output of the mineral elements. The alkali reserve was diminished.

In the discussion of the results of the previous experiment on acid feeding, reference was made to the possibility of injury to the tissue cells through the absorption of an excess of acid. The anæmia and the acid intoxication which developed after prolonged treatment in the first case were probably due to this cause. The data show, however, that relatively large quantities of hydrochloric acid can be ingested without harmful effects. Apparently the renal mechanism is able to continue the elimination of an excess of acid for lengthy periods, so that the alkali reserve though diminished, is not reduced to abnormally low levels. As might be expected, the deviations from the normal in both cases of tetany are in the opposite direction to those found in hyperplasia of the parathyroid glands, notably in Von Recklinghausen's disease of the skeleton, which is discussed in a later communication.

I am much indebted to Dr. John Gracie and Dr. David Campbell for their co-operation in this work.

SUMMARY.

1. A metabolic experiment has been carried out on a young subject, maintained exclusively on a milk diet affording a large mineral intake, to ascertain the effects of acid ingestion (HCl) on the utilization of the mineral elements.

2. As shown by the increase in the renal output of lime, magnesia, and phosphoric acid, and in the ratio of urinary to fæcal phosphorus, a higher degree of absorption of all the components of the mineral intake is induced.

3. The quantities retained are slightly diminished, which would appear to indicate that the process of calcification is adversely affected, presumably through the absorption of an excess of acid or of acid products.

4. From the increase in absorption and the fall in retention it is inferred that the administration of hydrochloric acid must tend to increase the concentration of the mineral elements in the blood.

5. The alterations in the mode of excretion of the mineral elements are discussed in relation to the changes involved in the readjustment of acid-base equilibrium. Under the conditions of the experiment the excess of phosphorus eliminated by the kidney after acid ingestion is derived from the intake and is primarily dependent on an increase in absorption; but it includes a small proportion of phosphorus which in normal circumstances, would be utilized in growth, probably with lime in the process of calcification.

6. The changes produced by the administration of hydrochloric acid in the treatment of post-operative tetany are described.

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Tucker-'60
Bib No. 462

J.A.M.A. 174:890-893. 1960.

Hydrochloric Acid Burns of the Stomach

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The ingestion of caustic acid produces effects on the gastrointestinal tract quite different from those caused by strong alkalis. Either acid or alkali usually results in a stomatitis and inflammatory reaction of the tongue and pharynx. In the case of alkalis, however, the major damage usually is in the esophagus; with acids it is usually in the stomach. Alkalis cause so much spasm on contact with the esophageal mucous membrane that they may not reach the stomach at all; if they do, they tend soon to be neutralized by the gastric acids and are unlikely to produce a lasting burn. Acids, on the other hand, are less damaging to the squamous cells lining the esophagus but set up severe reactions when they come in contact with the columnar epithelium of the stomach. Active muscular spasm is initiated in the stomach, particularly in the antrum, where the corrosive is retained long enough to cause prolonged damage.

The anatomic separation of the sites of maximum injury by alkalis and acids is statistical and by no means absolute. In reviewing 139 cases of pyloric stenosis resulting from acid ingestion, Gray and Holmes[1] found that 20% of the patients experi-

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enced esophageal stricture as well. They also pointed out that about 20% of patients who imbibe caustic alkalis develop strictures of the stomach as well as the esophagus.

Any one of the strong acids is capable of producing such changes. Perhaps the commonest offender, because the most easily obtained, is sulfuric acid. Such violent damage results that frequently the stomach is perforated and the patient dies of a fulminating peritonitis. Hydrochloric acid produces reactions which are somewhat less severe but which cause late scarring which necessitates surgical repair. Nitric acid may produce severe lesions, and marked damage has been reported from ingestion of a variety of salts, including ferrous sulphate, zinc chloride, calcium chloride, and calcium lactate.

Symptoms commence at intervals after ingestion according to the caustic properties of the agent. In the case of a strong acid there is immediate onset of burning of the mouth, frothing, and spitting, followed shortly by gagging and vomiting. The vomitus is likely to contain shreds of mucosa and to be bloody. The acute reactions persist for several days, then gradually subside. The patient, who at first must be nourished parenterally, improves to the point where he can take oral nourishment and may leave the hospital to continue his convalescence. Three or four weeks after the ingestion, however, vomiting recurs and becomes persistent. In most acid-induced strictures, surgical relief of the obstruction becomes mandatory.

A graphic description of the condition found by the surgeon was given in 1926 by Lord Moynihan. "In my own cases the pyloric antrum has presented the appearance of a normal uterus, the walls are thick and unyielding, the cavity small, and the pylorus projects downwards into the duodenum like the cervix into the vagina."[2]

Report of Cases

CASE 1.—A 23-month-old boy was admitted eight weeks after having drunk hydrochloric acid. At the time he had been examined by his physician, who noted a burn on the tip of his tongue but none in the mouth or pharynx. No treatment had been given, and the patient had not suffered further symptoms until 22 days later when he commenced to vomit. Vomiting had continued since then, about four times a day, anywhere from 15 minutes to two and one-half hours after meals. The vomitus was noted to contain mucus and undigested food. Roentgenograms showed a normal esophagus but a severe constriction of the body of the stomach (fig. 1), which was confirmed at laparotomy. A wedge resection of the stenosed segment was performed, and the patient made an uneventful recovery. The pathologist reported acute and chronic inflammation of the stomach, with scar formation and nodular hyperplasia of the smooth muscle of the cardia of the stomach. At the time of this writing, the patient has remained well and free of gastric symptoms for two years and seven months since discharge from the hospital.

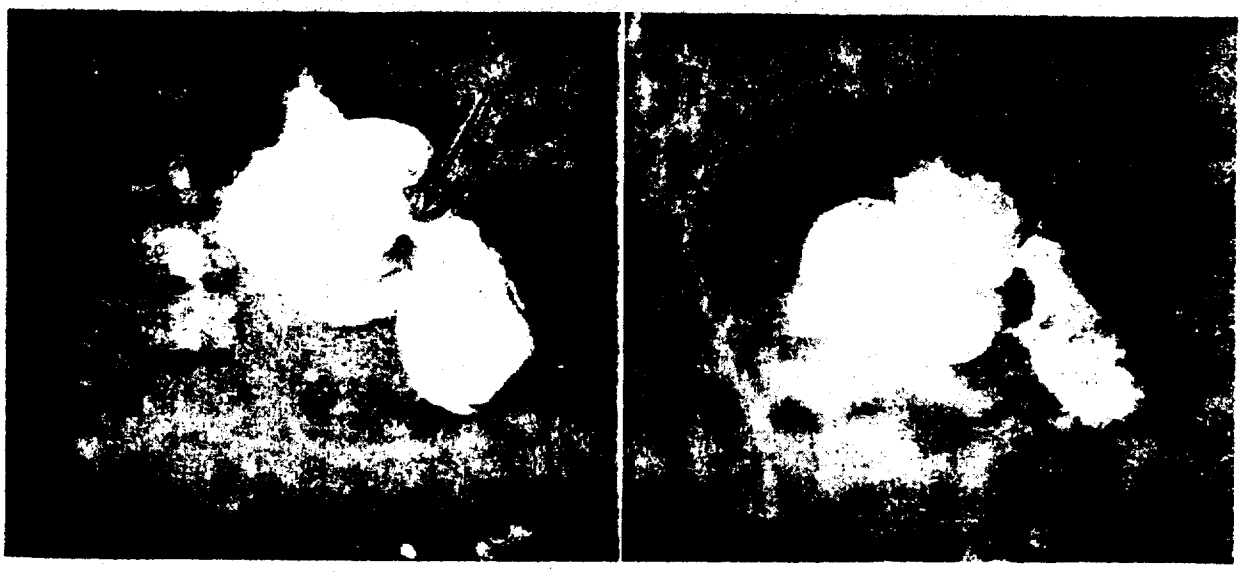

Fig. 1 (case 1).—Oblique and lateral views of the stomach two months after ingestion of hydrochloric acid. Arrows point to severe constriction of proximal part of stomach. Antrum also was constricted, and films indicate that gastric emptying was markedly delayed.

CASE 2.—A 4½-year-old boy found a glass of hydrochloric acid being used by plumbers at a construction site and drank an estimated 2-oz. (57 Gm.). He immediately ran to his home next door, smoking from his mouth and gagging. His mother gave him raw egg, which he immediately brought up. He was taken to the emergency ward of another hospital, where he arrived within one-half hour after his injury. He was observed there to be coughing and spitting up a brown material. Emergency treatment consisted of soda and milk by mouth and fluids given intravenously. His mother gave the information that he would drink almost anything and that a year before he had burned his mouth severe-

ly with iodine. He was transferred to Babies and Childrens Hospital, where he was observed to be acutely ill and lethargic, experiencing some respiratory distress, and having trickles of blood running out of the side of his mouth. Treatment included a tracheotomy, fluids given intravenously, streptomycin, penicillin, and a humid-air room. The patient responded and after three and one-half days commenced to take liquids by mouth.

Convalescence progressed, but the patient continued to vomit, particularly after eating solid foods. Esophageal dilations were carried out twice. Successive roentgenographic examinations showed progressive constriction of the distal part of the stomach (fig. 2). Finally, eight weeks after the injury, a laparotomy was performed and a partial gastric resection carried out. A gastroduodenostomy connected the pars media of the stomach to the dun-

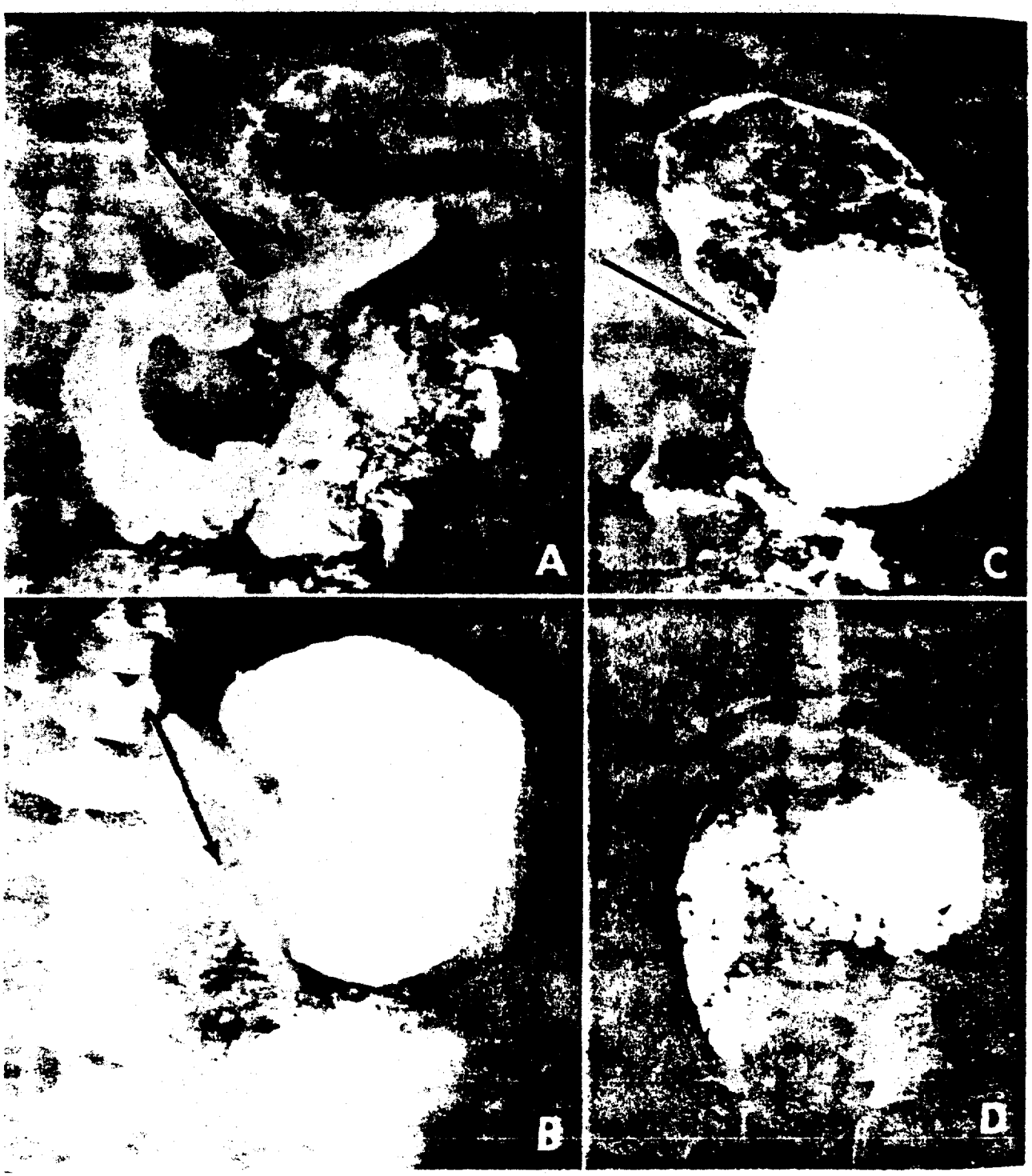

Fig. 2 (case 2).—Progressive constriction of gastric antrum (see arrows): *A*, three weeks, *B*, five weeks, and *C*, seven weeks after ingestion of hydrochloric acid. *C*, pyloric outlet is seen to be severely compromised. *D*, 24-hour film at seven weeks, showing that about 50% of meal remained in stomach one day after patient was given barium.

denum. The pathologist's report was acute gastritis with ulceration, fibrosis, and recent hemorrhage.

After discharge from the hospital, the patient's parents complained that he continued to vomit after meals. Four more hospital admissions ensued in the next 10 months, but no vomiting was observed while the boy was hospitalized. The parents were disinclined to accept a psychogenic basis for the vomiting and took the boy to other doctors. Elsewhere he had two more operations, said to be a resection of an esophageal stricture and an anastomosis of a segment of transverse colon to connect the esophagus to the stomach.

Summary

Acid ingested by a patient tends to produce its most severe damage in the stomach, whereas alkali affects the esophagus more markedly. Hydrochloric acid ingested by two children produced typical strictures of the stomach which necessitated surgical resection to relieve obstruction.

2065 Adelbert Rd. (6) (Dr. Tucker).

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VI. HISTOLOGICAL FINDINGS IN THE BRAIN IN THE CASE OF HYDROCHLORIC ACID INTOXICATION (ON THE BASIS OF AN OBSERVATION BY THE AUTHOR) [Histologische Belfunde im Hirn bei Salzsaure-vergiftung. (An Hand einer eigenen Beobachtung)]

by

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(Heidelberg Institute of Pathology, Dr. A. Schmincke, director)

from

Beit. z. path. Anat. u. allg. Path. 101 (1938) p. 60-65

Translation from German

VI. HISTOLOGICAL FINDINGS IN THE BRAIN IN THE CASE OF HYDROCHLORIC ACID INTOXICATION (ON THE BASIS OF AN OBSERVATION BY THE AUTHOR)

Carlheinz Velten

The pathology of acute, fatal hydrochloric acid intoxication has been summarized by Geissler. The findings concern primarily the local variations at the site of the direct action of the acid and their late sequelae. His data has been confirmed essentially by E. Petri, who adds toxic-degenerative processes in the liver, kidneys, and cardiac muscle as remote effects. The central nervous system has been studied very little in this direction. Occasionally, one finds that at the end of detailed organ study the remark that hyperemia and even small hemorrhages could be found in the brain, but that macroscopic findings were lacking.

For other caustic agents, the results of relatively numerous series of macroscopic and microscopic observations are available.

While for nitric acid vapors [Kamps], and for acetic acid [Barrancas, Neugebauer] hyperemia, stasis formation, and sometimes even massive ring hemorrhages have been described only macroscopically, Tagawa found in oxalic acid intoxication--along with similar macroscopic findings--severe changes in the ganglia cells as well as bloating of the cell bodies and their nuclei, decomposition of the Nissl's bodies, decomposition of the intracellular fibrils, and fatty degeneration of Purkinje's cells. For sulfuric acid intoxication, there are also the same changes in the ganglia cells, primarily in the area of the mamillary body and oliva. Takaya and Siki observed small foci of the necrosis with medula elimination in phosphoric acid intoxication, chromatolysis of the ganglia cells, and honeycomb degeneration. The same type of intoxication, Wertham describes

"ischemically changed" nerve cells in the second and third layer of the cerebrum and also in the cerebellum. In addition, there were extensive severe cell changes in both oliva with incrustation of the Golgi complexes and glia rosette formation in the vicinity of the oliva as well as fine-drop fatty degeneration of glia and ganglia cells. De Crinis mentions severe damage to the central nervous system for Lysol and sublimate intoxications. He found signs of edema, colliquations, disappearance of the tigroid lumps, proliferation of the satellite cells, bloating states in the ganglia cell bodies and their nuclei, and also neuronophagic processes. He regards the severe changes as the morphological reaction of the central nervous system to toxins circulating in the blood.

There are similar observations for caustic soda intoxications. Jankovich, Incze report acute severe swelling states in the ganglia cells, bloating of the nuclei, and shrinkage of the nuclear bodies, disappearance of the nucleus wall, shadow cell formation, neurono-axono-dendro phagic processes, and then homogenizing changes of the ganglia cells similar to the cell picture of ischemic cell disease. The findings sometimes developed diffusely and sometimes in the form of foci. For ammonia intoxications, the same findings are mentioned by Fazuka and Jankovich. In this case as well, there were severe degenerative changes in the ganglia cells along with fatty degeneration processes and the already mentioned peculiar homogenization processes in the histological picture. Fazuka also succeeded in the experimental production of the same severe changes in vessels, glia fibers, and ganglia cells. Orson mentions the following as the expression of a vital reaction of the central nervous system to intoxications of all types--bases, ammonia, acetic acid, CO, CO_2, luminal, strychnine,

alcohol, sublimate, arsenic, barium chloride, etc.--acute degenerative and coagulative processes of the ganglia cells. The pictures resemble each other to a large extent in the various intoxications.

Thus, it can be assumed as certain that just like the action, of other caustic agents, hydrochloric acid also does not represent merely a local caustic poison which leads to necrosis of the portions of the digestive tract which have into contact with the acid, but that damage as a result of remote toxic action occurs to the parenchymatic organs and even to the central nervous system through the remote actions which have already been mentioned.

On the occasion of a case of fatal hydrochloric acid poisoning which came to the Institute of Pathology for autopsy, I was able to observe a considerable pathological process in the ganglia cells and believe that I am able to provide a further contribution to the toxic action of hydrochloric acid on the central nervous system by reporting this case.

Case:* 48-year-old woman Sch., was admitted to the clinic in a high degree of shock, with imperceptible pulse, and with cold extremities, and with scale in the mouth and the oral cavity two hours after she had drunk 200 g household hydrochloric acid for the purpose of suicide. The stomach was pumped out in the hospital. Subsequent rinsing with milk. Initially good recovery, clear consciousness. Sudden nausea 10 hours after admission, death within a few minutes.

Autopsy findings: (Autopsy No. 10937, Obd. Dr. V.)

Extensive acid scaling of the severest degree of the esophagus in its lowest portion, of the stomach, and of the duodenum. Slight scaling of the upper portions of the esophagus. No findings in the other organs.

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* I am grateful to the Heidelberg Surgical Clinic (Prof. Kirschner) for the clinical data.

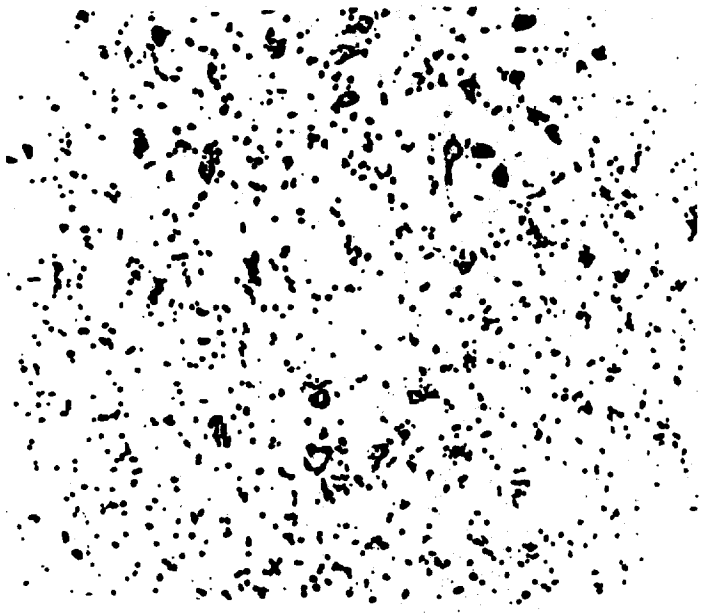

Fig. 1. Survey of the degenerative changes (swelling, satellite cell proliferation, pseudo-neuronophagia, neuronophagia).

Fig. 2. Acute cell pathology of Nissl's oliva band.

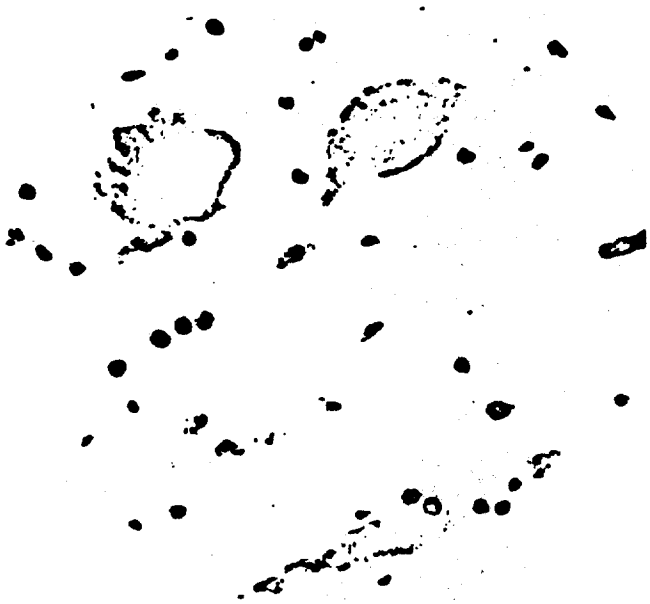

Fig. 3. Bloating of the cells, the cell nuclei, tigrolysis, disappearance of the cell membrane, dissolution of the ganglia cell (oliva band: 1/12 Olimm. Homal VI.

Fig. 4. Homogeneous coagulated changes (dome area).

Brain: Membranes without findings, likewise the convolutions. Contour compression at the bottom of the cerebellum, brain substance moist, without hemorrhages and softening. The ependyma of the cerebral cavity appears to be thickened slightly.

Spinal column: No findings on the membrane and on the cross section series.

Microscopic studies:

Stomach: Extensive scaling of the mucosa extending into the muscularis mucosae.

Brain: (Studies of pieces from various portions). Occasionally marked bloating of the cells, also of the nuclei, eccentric displacement of the nuclei, swelling of the dendrites, sometimes a fine grained decomposition of the cells with shrinkage of the cell bodies. Partially pronounced tigrolysis, shadow cell formation, pseudoneuronophagia and also genuine neuronophagia (Figs. 1, 2, and 3). Particularly in the dome area, noticable homogenization of the cytoplasm with complete disappearance of the structure. Coagulation processes were involved (Fig. 4). The coagulated cell protoplasm appears to be stained dark green in Nissl's preparation.

Spinal Column: The large motor ganglia cells also show swelling of the cell bodies in this case, eccentric displacement of the cell nuclei, and fine grained cell decomposition. Peculiar homogenization of the blood in the vessels as in the case of stasis.

Thus, acute, "severe", and homogenizing changes in the ganglia cells were found.

In the case of the former, pictures are involved such as occur in the case of acute Nissl's cell disease in various types of intoxication, infectious disease, and in burns. A vital occurrence can be assumed with

certainty for these. The homogenizing changes and likewise have been mentioned frequently. According to Orson, they represent the basic phenomenon of any vital reaction. However, their vital occurrence is still disputed, because one does not know the extent to which postmortal changes play a role in this. Recently, the same pictures have also been described elsewhere for Veronal intoxication (Scheidegger*).

It is difficult to say in what way one should conceive of these changes occurring. The following questions arise in the course of this:

1. Is a specific poison action involved?
2. Is a secondary occurrence present?

The similarity of the changes in the nerve cells is remarkable in the various types of intoxication. In my opinion, one would not go wrong if one were to assume that a specific toxic action is actually eliminated because of this. For it appears improbable that in different poisons, one and the same effect to the same organ systems can occur. This forces us to consider a secondary toxic effect. By means of this, the findings of all changes produced by caustic poison can be brought to a common denominator. The noxa originate in the body itself and therefore an autointoxication is involved. Perhaps we should seek the explanation for the secondary toxic damage which differs from the original toxic effect but is nevertheless caused by it in a parallel with the findings in a case of burns and scaldings. Relatively large areas of skin are coagulated as in this case, and the absorbed decomposition substances cause severe toxic effects; thus, in the case of acid scaling of the mucosa in the digestive tract, protein decomposition products occur in loco which are absorbed by the blood and lymph vessels of the lower abdominal wall layers and supplied to the organism

* I have not seen anything similar in the case of dimethyl aminophenazone intoxication.

as toxins. They lead to the severe changes of the ganglia cells.

Thus, an absorptive protein autointoxication is involved. The same degradation products must naturally also be held responsible for the damage to the parenchymatous organs.

The degradation products also occur in the course of mild, nonfatal hydrochloric acid intoxications following only slight coagulation processes of the mucosa. They then probably lead to acute swelling processes and degenerative phenomena in the ganglia cells. As a result of the weaker toxic action in the case of the lesser anatomical changes, only temporary damage to the ganglia cells, which is reversible, results from this.

The sudden occurrence of death after initial improvement is a proof for the fact that the degenerative changes in the ganglia cells take a certain time until they have an effect and the functions of the cells are extinguished.

In the case of the question of the different localization of the changes in the brain, one can think of the fact that individual portions have a stronger affinity for the protein degradation products, and perhaps a pathoclisis is therefore involved. However, nothing more certain can be said. For particularly preferred locations (dome, thalamus, oliva), the anatomical distribution of the vessels, that is the vascular factor, could also play a role. We were concerned in demonstrating the findings of a homogenizing, coagulating, and severe degenerative change of the ganglia cells in acute fatal hydrochloric acid intoxication in particular. The same changes have been described in other caustic agent intoxications. The same effect in the course of different conditions permits drawing the conclusion that there is a common cause. The tissue scaling which is the same in the case of the different intoxications can be held responsible

for this. The damage to the ganglia cells would therefore be the result of a protein autointoxication due to the uptake of dead, decomposed protein substances.

Velten-'38
Bib No. 471

Beit. Z. Path. Anat. U. Allg. Path. 101:60-65.

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VI.
Histologische Befunde im Hirn bei Salzsäurevergiftung.
(An Hand einer eigenen Beobachtung.)

Von
Carlheinz Velten.

Aus dem Pathologischen Institut Heidelberg.
(Vorstand: Prof. Dr. A. Schmincke.)

Mit 4 Abbildungen im Text.

(Eingegangen am 28. November 1937.)

Die Pathologie der akuten, tödlichen Salzsäurevergiftung ist von GEISSLER zusammenfassend dargestellt. Die Befunde betreffen vor allem die lokalen Veränderungen am Ort der unmittelbaren Säurewirkung und ihre Spätfolgen. Seine Angaben werden im wesentlichen von E. PETRI bestätigt und ergänzend toxisch-degenerative Prozesse an Leber, Nieren und am Herzmuskel als Fernwirkung angeführt. Das Zentralnervensystem ist in dieser Richtung noch wenig untersucht. Vereinzelt findet man am Schluß eingehender Organuntersuchungen die hinweisende Bemerkung, daß sich im Hirn Hyperämie, auch kleine Blutaustritte finden ließen; mikroskopische Befunde dagegen fehlen.

Für andere Ätzmittel liegen die Ergebnisse einer größeren Reihe von makro- wie mikroskopischen Beobachtungen vor.

Während für salpetrigsaure Dämpfe (KAMPS) und für Essigsäure (BARRANCAS, NEUGEBAUER) auch nur makroskopisch Hyperämien, Stasenbildung, teilweise auch massenhaft Ringblutungen beschrieben sind, fand TAGAWA bei Oxalsäurevergiftung neben ähnlichen makroskopischen Befunden schwere Veränderungen an den Ganglienzellen, wie Blähung der Zelleiber und ihrer Kerne, Zerfall der NISSL-Schollen, Zerfall der intracellulären Fibrillen, auch Verlettungen von PURKINJE-Zellen. Dort sind auch für Schwefelsäurevergiftung gleiche Veränderungen an den Ganglienzellen, vornehmlich im Bereich von Corpus mamillare und Olive erwähnt. TAKAYA und SIKI sahen bei Phosphorsäurevergiftung Nekroseherdchen mit Markausfall, Chromatolyse der Ganglienzellen und wabige Degeneration. WERTHAM beschreibt bei der gleichen Vergiftungsart in der 2. und 3. Großhirnrindenschicht, ebenso im

Kleinhirn „ischämisch-veränderte" Nervenzellen. Wenn [illegible] in beiden Oliven durchgehende schwere Zellveränderungen mit Inkrustation der GOLGI-Netze und Gliarosettenbildung in der Umgebung der Oliven, sowie feintropfige Verfettung von Glia- und Ganglienzellen zu beobachten. DE CRINIS führt für Lysol- und Sublimatvergiftungen schwere Schädigungen am Zentralnervensystem an. Er fand Verflüssigungserscheinungen — Kolliquationen —, Schwund der Tigroidschollen, Trabantzellvermehrung, Blähungszustände an Ganglienzellleibern und ihren Kernen, auch neuronophagische Prozesse. Er sieht in diesen schweren Veränderungen die morphologische Reaktion des Zentralnervensystems auf im Blut kreisende Giftstoffe.

Für Laugenvergiftungen liegen ähnliche Beobachtungen vor. JANKOVICH-IKCZÉ berichten über akute schwere Schwellungszustände an den Ganglienzellen, Blähung der Kerne, Schrumpfung der Kernkörperchen, Schwund der Kernwand, Zellschattenbildung, neurono-axono-dendrophagische Prozesse, dann homogenisierende Veränderungen der Ganglienzellen ähnlich dem Zellbild der ischämischen Zellerkrankung. Die Befunde waren teils diffus, teils herdförmig zur Entwicklung gekommen. Für Salmiakgeistvergiftungen werden von FAZEKAS und JANKOVICH die gleichen Befunde erwähnt. Auch hier beherrschen schwere degenerative Veränderungen an den Ganglienzellen neben Verfettungsprozessen und den schon erwähnten eigenartigen Homogenisierungsvorgängen das histologische Bild. FAZEKAS gelang auch die experimentelle Erzeugung gleichschwerer Veränderungen an Gefäßen, Gliafasern und Ganglienzellen. ORSOS erwähnt als Ausdruck einer vitalen Reaktion des Zentralnervensystems auf Vergiftungen aller Art — Laugen, Ammoniak, Essigsäure, CO, CO_2, Luminal, Strychnin, Alkohol, Sublimat, Arsen, Bariumchlorid usw. — akute, degenerative und koagulierende Prozesse an den Ganglienzellen. Die Bilder ähnelten sich bei den verschiedenen Vergiftungen weitgehend.

Es kann somit als sicher angenommen werden, daß ähnlich der Wirkung anderer Ätzmittel auch die Salzsäure nicht nur ein lokales Ätzgift darstellt, das zur Nekrose an mit Säure in Berührung gekommenen Teilen des Verdauungstraktus führt, sondern daß über die bereits erwähnten Fernwirkungen an den parenchymatösen Organen hinaus, auch im Zentralnervensystem Schädigungen als Folge einer Giftfernwirkung entstehen.

Gelegentlich eines im Pathologischen Institut zur Sektion gekommenen Falles von tödlicher Salzsäurevergiftung konnte ich einen erheblichen Erkrankungsprozeß der Ganglienzellen beobachten und glaube durch seine Mitteilung einen weiteren Beitrag zur Giftwirkung der Salzsäure auf das Zentralnervensystem liefern zu können.

Fall [1]): 48jähr. Frau Sch. wurde, nachdem sie zum Zwecke des Suicids 2 Stunden vor Einlieferung 200 g Haushaltsalzsäure getrunken hatte, hochgradig schockiert, mit unfühlbarem Puls und kalten Extremitäten, mit Schorf am Mund und in der Mundhöhle in die Klinik eingeliefert. Hier Magenspülung, Nachspülung mit Milch. Zunächst gute Erholung, klares Bewußtsein. 10 Stunden nach Einlieferung plötzliche Übelkeit. Tod in wenigen Minuten.

[1]) Die klinischen Angaben verdanke ich der Chir. Klinik Heidelberg (Prof. KIRSCHNER).

Sektionsbefund: (S.N. 109/37. Obd. Dr. V.).

Ausgedehnte Säureverschorfung schwersten Grades des Ösophagus in seinem untersten Teil, des Magens und des Duodenums. Geringe Verschorfung

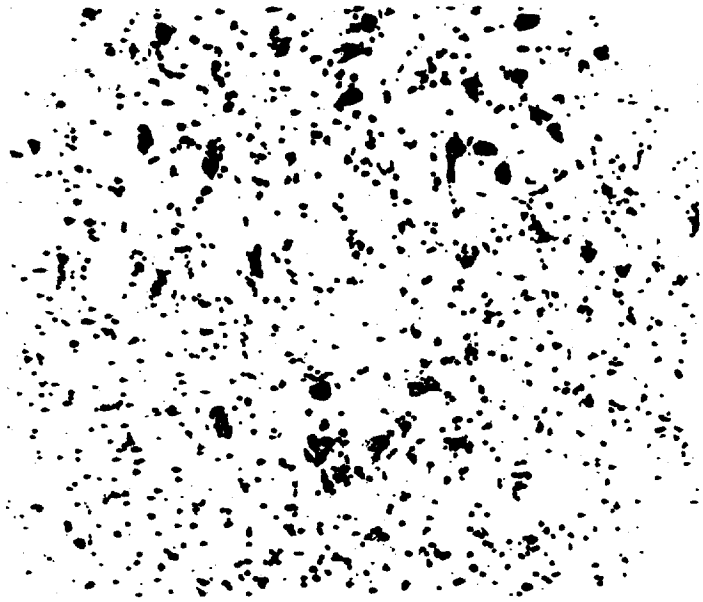

Abb. 1. Übersicht über degenerative Veränderungen (Schwellung, Trabantzellvermehrung, Pseudoneuronophagie, Neuronophagie).

Abb. 2. Akute Zellerkrankung Nissls (Olivenband).

der oberen Teile des Ösophagus. An den übrigen Organen kein Befund.

Hirn: Häute o. B., ebenso Windungen. Konturkompression unten am Kleinhirn. Hirnsubstanz feucht, ohne Blutungen und Erweichungen. Das Ependym der Hirnhöhlen scheint geringgradig verdickt.

Rückenmark: An den Häuten und auf der Querschnittsserie nichts Besonderes.

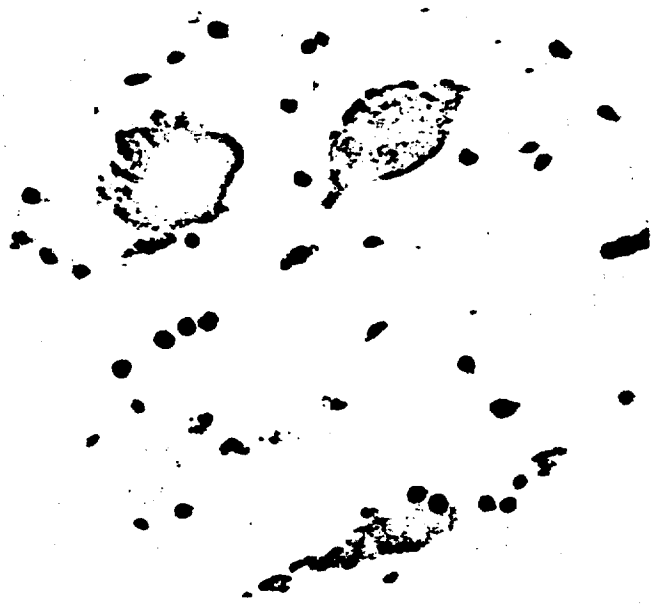

Abb. 3. Blähung der Zelle, der Zellkerne, Tigrolyse, Schwund der Zellmembran. Auflösung der Ganglienzelle (Olivenband). 1/12 Ölimm. Homal VI.

Mikroskopische Untersuchungen:

Magen: Ausgedehnte Verschorfung der Schleimhaut bis in die Muscularis mucosae reichend.

Hirn: (Untersuchungen von Stücken aus verschiedenen Teilen). Z. T. starke Blähung der Zellen, auch der Kerne, exzentrische Verlagerung der Kerne, Schwellung der Dendriten, z. T. feinkörniger Zerfall der Zellen unter Schrumpfung der Kernkörperchen. Teilweise ausgesprochene Tigrolyse. Zellschattenbildung. Pseudoneuronophagie, auch echte Neuronophagie (Abb. 1, 2, 3). Besonders im Haubengebiet auffällige Homogenisierung des Cytoplasmas mit vollständigem Strukturschwund. Es

Abb. 4. Homogenisierende, koagulierende Veränderungen (Haubengebiet).

handelt sich hier um Prozesse der Koagulation (Abb. 4). Das koagulierte Zellprotoplasma erscheint im NISSL-Präparat dunkelgrünlich verfärbt.

Rückenmark: Die großen motorischen Ganglienzellen zeigen auch hier Schwellung der Zelleiber, exzentrische Verlagerung des Zellkerns, feinkörnigen Zellzerfall. In den Gefäßen eigenartige Homogenisierung des Blutes, wie bei Stase.

Gefunden wurden also akute, „schwere" und homogenisierende Veränderungen an den Ganglienzellen.

Bei den ersteren handelt es sich um Bilder, wie sie als akute Zellerkrankung NISSLS bei den verschiedensten Vergiftungsarten, bei Infektionskrankheiten, auch bei Verbrennungen vorkommen. Für sie dürfte eine vitale Entstehung als sicher angenommen werden.

Die homogenisierenden Veränderungen sind ebenfalls schon mehrfach erwähnt worden. Sie stellen nach OSSIS das Grundphänomen jeder vitalen Reaktion dar. Ihre vitale Entstehung ist aber noch strittig, weil man nicht weiß, inwieweit postmortale Veränderungen dabei eine Rolle spielen. Neuerdings sind die gleichen Bilder auch für Veronalvergiftung von anderer Seite beschrieben (SCHEIDEGGER)[1].

Es ist schwer zu sagen, auf welche Weise man sich diese Veränderungen entstanden denken soll. Es ergeben sich dabei folgende Fragen:

1. Handelt es sich um eine spezifische Giftwirkung?
2. Liegt ein sekundäres Geschehen vor?

Auffallend ist die Gleichheit der Veränderungen an den Nervenzellen bei den verschiedenen Vergiftungsarten. Man geht m. E. nicht fehl, wenn man annimmt, daß damit eigentlich eine spezifische Giftwirkung ausscheidet. Denn es erscheint unwahrscheinlich, daß bei verschiedenen Giftmitteln ein und dieselbe Wirkung an den gleichen Organsystemen entstehen kann. Das bringt uns dazu, eine sekundäre Giftwirkung anzunehmen. Damit lassen sich die Befunde aller durch Ätzgifte hervorgerufenen Veränderungen auf einen gemeinsamen Nenner bringen. Die Noxen sind im Körper selbst entstanden, es handelt sich also um eine Autointoxikation. Die Erklärung dieser sekundären, von der ursprünglichen Giftwirkung verschiedenen, aber doch durch sie bedingten Giftschädigungen möchten wir in einer Parallele mit den Befunden bei Verbrennungen und Verbrühungen suchen. Wie hier größere Hautbezirke koaguliert werden und die resorbierten Zerfallsstoffe schwere Giftwirkungen hervorrufen, so entstehen bei der Säureverschorfung der Schleimhaut des Verdauungstraktus in loco Eiweißabbauprodukte, die durch Blut- und Lymphgefäße der tieferen Magenwandschichten aufgenommen und dem Organismus als Giftstoffe zugeführt werden. Sie führen zu den schweren Ganglienzellveränderungen.

Es handelt sich also um eine resorptive Eiweißautointoxikation. Die

[1] Bei Dimethylaminophenazonvergiftung habe ich etwas Derartiges nicht gesehen.

gleichen Abbauprodukte sind natürlich auch für die Schädigungen an den parenchymatösen Organen verantwortlich zu machen.

Die Abbauprodukte entstehen auch bei leichten, nicht tödlichen Salzsäurevergiftungen im Anschluß an nur geringe Koagulationsprozesse der Schleimhaut. Sie führen dann wohl zu akuten Schwellungsprozessen und Entartungserscheinungen an den Ganglienzellen. Infolge der schwächeren Giftwirkung bei den geringeren anatomischen Veränderungen entstehen daraus aber nur temporäre Schädigungen der Ganglienzellen, die reversibel sind.

Das plötzliche [illegible]tzen des Todes nach anfänglicher Besserung ist ein Beweis dafür, da[illegible] degenerativen Veränderungen an den Ganglienzellen eine gewisse [illegible] beanspruchen, bis sie sich auswirken, und die Funktion der Zell[illegible]scht.

Bei der Frag[illegible]h der verschiedenartigen Lokalisation der Veränderungen im Hi[illegible]n man daran denken, daß einzelne Teile eine stärkere Affinität zu den [illegible]ßabbauprodukten haben, vielleicht handelt es sich also um eine Pat[illegible]se. Etwas Sicheres läßt sich aber nicht sagen. Für besonders bevorzug[illegible]stellen (Haube, Thalamus, Olive) könnte auch die anatomische Verteilun[illegible]er Gefäße, also der vasale Faktor, eine Rolle spielen.

Es kam [illegible] darauf an, die Befunde einer homogenisierenden koagulierenden [illegible] und schweren degenerativen Veränderung an den Ganglienzellen [illegible]i akuter, tödlicher Salzsäurevergiftung besonders herauszustellen. Bei anderen Ätzmittelvergiftungen sind die gleichen Veränderungen beschrieben worden. Diese gleiche Wirkung bei verschiedenen Bedingtheiten läßt auf eine gemeinsame Ursache schließen. Hierfür kann man die bei den verschiedenen Vergiftungen gleiche Gewebsverschorfung verantwortlich machen. Die Ganglienzellschädigung wäre damit also die Folge einer Eiweißautointoxikation durch Aufnahme zugrunde gegangener, abgebauter Eiweißkörper.

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J. Appl. Physiol. 32(5):585-590. 1972.
JOURNAL OF APPLIED PHYSIOLOGY
Vol. 32, No. 5, May 1972. Printed in U.S.A.

Winship-'72
Bib. no. 491

Acid loss in the human duodenum

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WINSHIP, DANIEL H., CARLTON R. CAFLISCH, AND WILLIAM J. SCHULTE. *Acid loss in the human duodenum.* J. Appl. Physiol. 32(5): 585-590. 1972.—The disposal of acid by the human duodenum was investigated by infusing polyethylene glycol-marked HCl into the proximal duodenum at controlled but varying rates, and sampling of fluid and recording pH distally. Correction was made for gastric reflux. The net gain of fluid into the duodenum increased linearly with the acid (but not saline) infusion rate. The rate of H^+ loss in the segment was not a linear function of the acid infusion rate, however, but increased with a decreasing increment as the acid infusion rate increased, suggesting that the process of acid disposal is a saturable one. In some studies, sampling carried out from two points in the duodenum showed that dilution and neutralization of acid by pancreatic juice and bile in the proximal segment was limited, but further H^+ loss occurred in the distal segment, linearly related to the rate of acid entry into the segment. The H^+ loss in the distal segment proceeded whether there was net gain or loss of fluid in the segment, suggesting either H^+ absorption or further entry of HCO_3^- into the duodenal lumen to react with the H^+ present.

duodenal fluid; neutralization; acid infusion; pH recording; osmolality; bicarbonate; polyethylene glycol

HYDROCHLORIC ACID, on entering the duodenum, stimulates the flow of bicarbonate-rich pancreatic juice (2), bile (10), and Brunner's gland juice (5), all of which dilute and neutralize the acid. The physiologic importance of this process is implied by the frequency and rapidity with which duodenal mucosal ulceration develops in conditions such as the Zollinger-Ellison syndrome (15) and the Mann-Williamson preparation (8). In these situations, a neutral pH in the duodenum is not maintained, due primarily to an excess of acid entering the duodenum in the Zollinger-Ellison syndrome and to diversion of the bile and pancreatic juice in the Mann-Williamson preparation.

Few investigations of the capacity of the human duodenum to dispose of acid have been reported. The available information indicates, however, that up to 20 ml fluid/min may be secreted into the duodenum in response to the instillation of hydrochloric acid (12), that up to 0.9 mEq HCl/min may be neutralized (4), and that the neutralization process is a precise one (7).

We have investigated the problem of acid loss in the intact human duodenum by infusing acid into the proximal duodenum at various rates and recording pH and analyzing fluid aspirated distally.

METHODS

Subjects. Eleven men aged 28–60 years were studied. None showed evidence of gastrointestinal, hepatic, or pancreatic disease.

Technique. A four lumen polyvinyl tube (each lumen 1.8 mm internal diameter) was constructed and fused longitudinally to the lead of an intragastric pH electrode (Beckman 39042) (Fig. 1). The infusion tip (I), rendered radiopaque by a metal marker, was placed (and frequently monitored) fluoroscopically just beyond the duodenal bulb, 1 or 2 cm into the descending portion of the duodenum. An aspiration tip (A_1), also with a radiopaque marker, opened 15 cm distal to I and lay in the proximal half of the third portion of the duodenum. The bulb of the glass electrode (GE), 30 cm distal to I, was therefore at or just beyond the ligament of Treitz. A second aspiration tip (A_2) was at the level of the glass electrode, and the tubing constituting A_2 was fashioned into a small basket around the electrode to prevent spurious pH readings resulting from contact between electrode and mucosa (3). The fourth lumen (R) opened adjacent to the electrode bulb. This lumen, filled with saturated KCl, was connected externally to a reservoir of the same solution into which a calomel half-cell was placed, and served as the pH reference electrode. The distal tip of the reference tube was plugged with an asbestos fiber wick that permitted electrical contact but prevented free flow of the solution into the intestinal lumen. The electrical leads were connected to a Beckman model 76 pH meter and pH was recorded continuously on a Beckman potentiometric recorder.

Two infusion solutions were used: isotonic NaCl (pH 6.30 ± 0.30) and 0.1 N HCl, made isotonic with NaCl (pH 1.08 ± 0.06). Each contained 1% polyethylene glycol (PEG, mol wt 4,000) as a nonabsorbable marker.

With the subject in the left lateral decubitus position, test solution was infused with a Bowman constant-infusion pump, gastric aspiration was performed by machine and hand through a 16 F levin tube placed fluoroscopically in the gastric antrum, and duodenal samples were obtained by a constant withdrawal pump or by hand aspiration at a rate of 0.5–1 ml/min from each tip.

Preliminary studies showed that infused acid reached the pH electrode within 90 sec after the start of the infusion, regardless of the infusion rate employed. The onset of the neutralizing reaction was evident within 3–5 min after beginning infusion; within 5–7 min after the start of infusion the pH recording stabilized. After an equilibration

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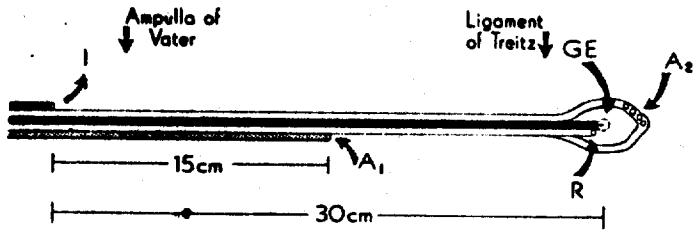

FIG. 1. Tube-glass electrode assembly. I = infusion tip; A_1, A_2 = aspiration tips; GE = glass electrode; R = reference electrode bridge. When electrode assembly is positioned in the duodenum, localization of pylorus, ampulla of Vater, and ligament of Treitz are approximately as indicated by arrows.

period of 15 or 30 min, one or two 15-min study periods were carried out. Infusions at three or four different rates were done during each study, always progressing from low to high rates. The infusion rates were from 1 to 23 ml/min. When a change of infusion solution from saline to acid was carried out, the next equilibration period was always 30 min in duration. All saline infusions were performed before acid was infused.

Volumes of samples were measured and titratable acidity ($[H^+]$) was determined by electrometric titration with 0.1 N NaOH to pH 7. PEG concentration ([PEG]) was measured by the turbidometric method of Hyden (6); osmolality was determined with an Advanced Instruments osmometer.

The rate of duodenogastric reflux of the infused solution was calculated from the volume of gastric aspirate and the PEG concentration in gastric aspirate and infusion solution. The corrected infusion rate (CIR_I) for each period was determined by subtracting reflux rate from the infusion pump rate. A mean of 12% of the infused acid was lost by reflux; the reflux rate ranged from 0 to 42%. Owing to variations in reflux rate when two sequential study periods were performed at the same pump speed, the corrected infusion rates for the two periods almost invariably differed. Thus each study period was calculated individually and considered as a separate datum.

In the initial set of studies, the proximal aspiration tip, A_1, was not utilized. The net changes in fluid volume and acid loss between the infusion point, I, and A_2 were calculated as follows:

Duodenal flow rate at A_2 (DFR_{A_2}) (ml/min):

$$DFR_{A_2} = \frac{CIR_I \cdot [PEG]_I}{[PEG]_{A_2}} \qquad (1)$$

Change in net flow rate between I and A_2 ($\Delta F_{I \to A_2}$) (ml/min):

$$\Delta F_{I \to A_2} = DFR_{A_2} - CIR_I \qquad (2)$$

Rate of H^+ disappearance between I and A_2 ($\Delta H_{I \to A_2}$) (mEq/min):

$$\Delta H^+_{I \to A_2} = \frac{(CIR_I \cdot [H^+]_I) - (DFR_{A_2} \cdot [H^+]_{A_2})}{1{,}000} \qquad (3)$$

The pH records were analyzed by calculating the percent of time that the recorded pH was above each pH increment from 1 through 8. From this analysis, pH-percent-of-time graphs were constructed as described by Bircher et al (3).

Subsequent studies were performed to observe in greater detail some of the characteristics of H^+ disappearance. In these subsequent studies, simultaneous aspirations were carried out from A_1 and A_2. The duodenum was therefore divided into two 15-cm study segments. The proximal segment, I to A_1, received the inflow from pancreatic and common bile ducts. If we assume adequate mixing of these fluids and the infusion fluid in the segment, the sample aspirated at A_1 then reflected net changes in volume and acidity occurring between I and A_1. The calculations for these changes are exactly as before, except that A_2 is replaced by A_1 in the three equations.

The distal 15-cm segment, A_1 to A_2, was then perfused by the fluid flowing past A_1. Further changes in volume or acidity occurring in this segment were attributed only to absorption or secretion across the duodenal mucosa.

Calculation of the rate at which fluid entered the distal segment was performed by subtracting the aspiration rate at A_1 from DFR_{A_1}. The resulting value was termed CIR_{A_1}. The rate at which acid flowed into the distal segment (mEq/min) was thus determined by:

$$\frac{CIR_{A_1} \cdot [H^+]_{A_1}}{1{,}000} \qquad (4)$$

With these data and the values for $[PEG]_{A_2}$ and $[H^+]_{A_2}$, changes in flow rate and acid content between A_1 and A_2 were calculated.

Standard statistical methods were utilized to determine regressions, correlations and significance of differences (11).

RESULTS

Total 30-cm segment. The acid solution was infused during 76 study periods. Increasing rates of acid entering the duodenum evoked an increase in flow rate at the distal aspiration tip. The net addition of fluid, although linearly related to the rate of acid infusion, increased at a lower rate than did the acid infusion rate (Fig. 2A). This difference is reflected by the slope of the linear regression line of 0.618. The regression slope is significantly different from zero ($P < 0.001$) and intersects the ordinate at 1.62 ml/min. (This value for Y is not significantly different from zero [$0.10 < P < 0.20$], however, so no inference may be safely drawn from it regarding the magnitude of basal, unstimulated endogenous flow into the duodenum.)

The analysis of regression and correlation for individual studies, represented by the fine lines in Fig. 2A, are shown in Table 1. The mean correlation coefficient of 0.89 (Fig. 2A) is reduced to 0.78 if correlation of all points is calculated without separation into individual studies.

Normal saline was infused during an additional 27 study periods (Fig. 2B). At least two saline periods were carried out in each subject, all prior to the infusion of acid. The regression slope does not differ significantly from zero ($P < 0.4$) indicating that there was no measurable response to the saline infusion. The regression line for saline intersects the ordinate at 0.97 ml/min. (This value for Y does differ significantly from zero [$0.005 < P < 0.01$] and may reflect the magnitude of unstimulated flow in the duodenum.)

Titration data were available from 40 of the 76 acid infusion periods, from eight of the subjects. Figure 3 represents the calculated rate of H^+ loss between I and A_2 as a

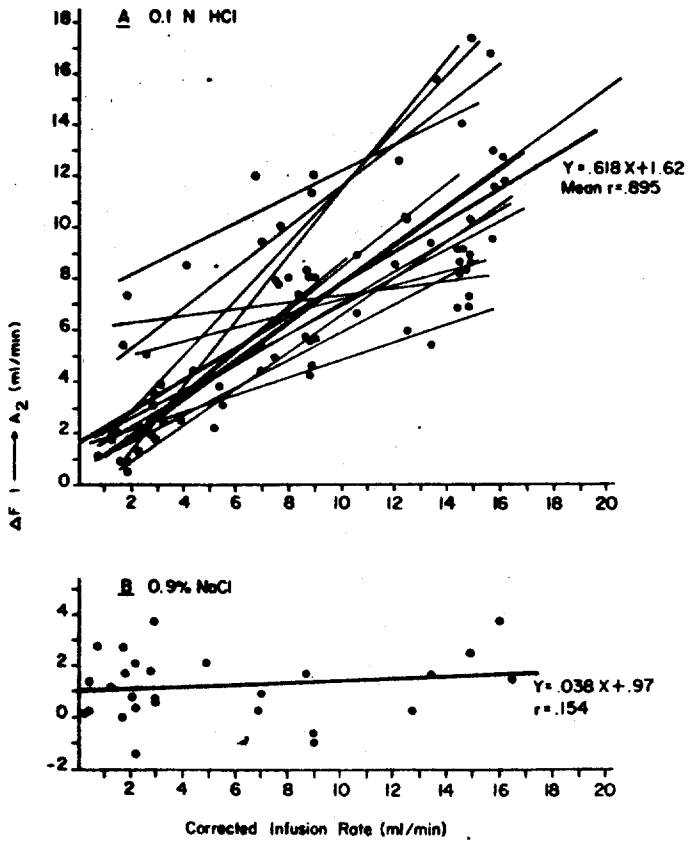

FIG. 2. Flow evoked by acid and saline infusions. *A:* net change in flow rate (ΔF) between infusion tip (I) and distal aspiration tip (A_2) in response to HCl infusions; acid infusions cause increased flow. *B:* flow does not increase in response to saline infusions.

TABLE 1. *Analysis of regression and correlation of individual studies shown in Fig. 1*

Patient (Study)	No. of HCl Study Periods	Linear Regression Equation	Correlation Coef (r)
1	8	$Y = 0.733X + 0.34$	0.988
2	8	$Y = 0.872X - 0.21$	0.858
3(*a*)	5	$Y = 0.783X + 3.67$	0.986
(*b*)	4	$Y = 1.270X - 1.28$	0.983
4(*a*)	6	$Y = 0.708X - 0.53$	1.000
(*b*)	4	$Y = 0.539X + 1.48$	0.900
5(*a*)	6	$Y = 0.130X + 6.03$	0.347
(*b*)	4	$Y = 0.500X + 7.13$	0.934
6(*a*)	7	$Y = 0.525X + 0.60$	0.981
	3	$Y = 0.740X + 0.42$	0.945
7	7	$Y = 0.264X + 4.47$	0.765
8	5	$Y = 0.809X + 0.26$	0.983
9	3	$Y = 0.338X + 1.45$	0.891
10	3	$Y = 1.202X - 0.54$	0.929
11	3	$Y = 0.594X + 1.02$	0.885
			Mean r 0.895

function of the corrected H^+ infusion rate, and indicates a curvilinear relationship.

In 66 of the 76 acid perfusion periods the pH was continuously recorded at the ligament of Treitz. In the remaining 10 study periods, malfunction of the pH meter or damage to the pH electrode with resultant short circuiting prevented pH measurements. For analysis of pH-time relationships, neutralization to pH 4.0 was arbitrarily selected as an example for illustration (Fig. 4). During all periods with corrected infusion rates of less than 0.9 mEq HCl/min, the pH at the ligament of Treitz remained above 4.0 over 76 percent of the time. At higher infusion rates, however, the neutralization ability began to be overcome. Similar analyses for other pH levels showed, for example, that the pH was always maintained above 1.0 during all infusions; pH was maintained above 2.0 over 88% of the time at corrected infusion rates up to 1.2 mEq/min, but at that or higher rates, neutralization was no longer as uniformly sustained.

During the saline infusions, the pH at the ligament of Treitz was always above 6.0.

Two 15-cm segments. Fifty acid infusion periods and eighteen saline infusion periods were studied in eight of the

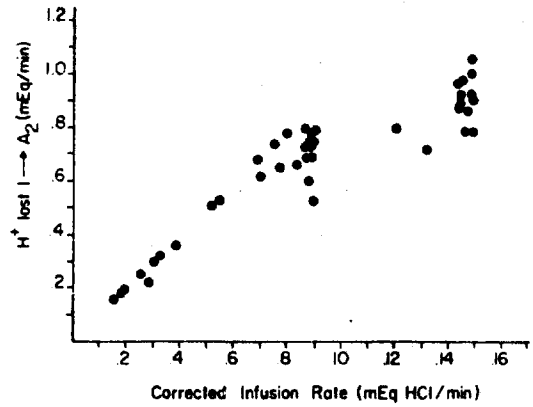

FIG. 3. Acid loss in entire duodenum. Net H^+ loss measured over 30-cm duodenal segment ($I \rightarrow A_2$) shows a curvilinear relationship to acid infusion rate.

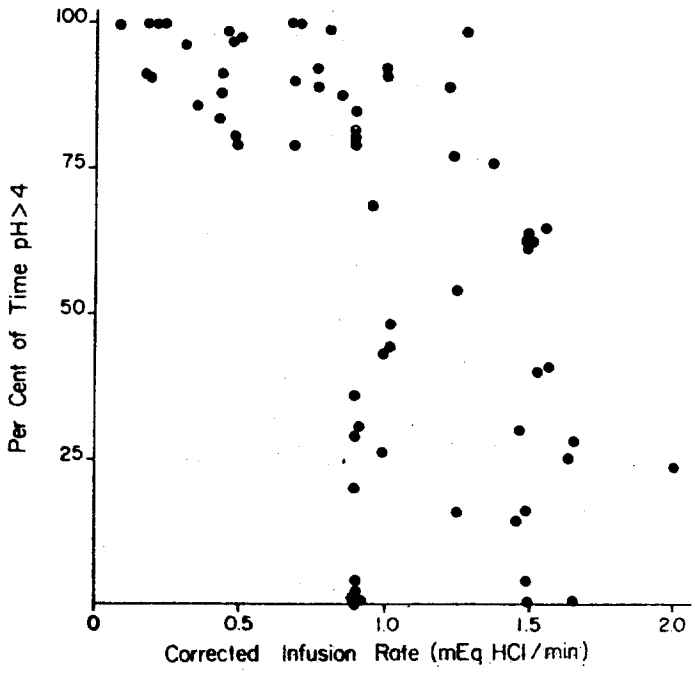

FIG. 4. Adequacy of acid disposal. From glass electrode records, intraduodenal pH at the ligament of Treitz was used to calculate fraction of time the pH was above each pH unit increment; here result is shown utilizing pH 4 as an example.

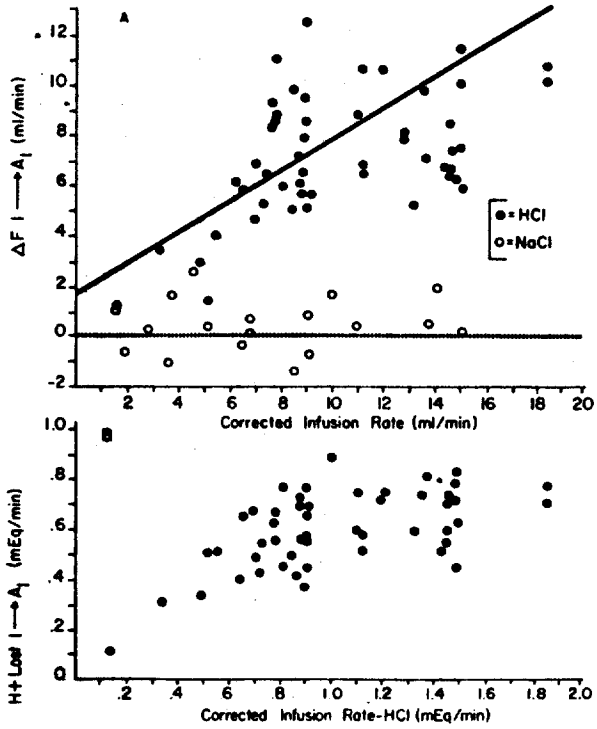

FIG. 5. *A:* net change in flow rate (ΔF) in proximal study segment ($I \rightarrow A_1$) in response to different acid and saline infusion rates. Heavy line is mean regression line of Fig. 2*A*, shown for comparison. Dashed line represents zero value for ΔF. *B:* H^+ loss from the proximal study segment ($I \rightarrow A_1$) as a function of H^+ infusion rate.

subjects. Pump rates ranged from 2 to 23 ml/min; aspirations were carried out simultaneously from A_1 and A_2. As depicted in Fig. 5*A*, the net rate of fluid gain in the proximal segment increased with increasing acid infusion rates up to infusion rates of slightly below 10 ml HCl/min; then the rate of fluid addition from endogenous sources increased no further even with higher acid infusion rates. Saline infusion evoked no intraluminal fluid gain. Acid loss from this segment followed a pattern similar to that for fluid gain (Fig. 5*B*).

In the distal segment, a further net gain of fluid into the lumen occurred, linearly related to the amount of acid entering the segment (Fig. 6*A*). Gain of fluid into the lumen occurred, however, only with rates of acid entry into the segment of 0.2 mEq/min or greater. At acid infusion rates below 0.2 mEq/min, net loss of fluid from the segment occurred.

Acid loss from the distal segment was also related to the rate of acid infusion into the segment at A_1 (Fig. 6*B*) and proceeded whether fluid was being lost from the lumen or secreted into it (Fig. 6*A*).

Measurements of osmolality were performed on samples from 17 acid infusion study periods and eight saline infusion periods in four of the subjects. The fluid at A_1 invariably showed a decrease in osmolality relative to infused fluid during acid (not saline) infusions; the loss was partially regained during passage through the distal study segment (Fig. 7).

DISCUSSION

Several factors contribute to the reduction of acid concentration within the duodenum. These include neutralization and dilution by the addition of endogenous alkalin fluids and, possibly, some loss of H^+ through the duodena mucosa (7). Our study provides information on the ne result of all these factors by assessing changes in HC

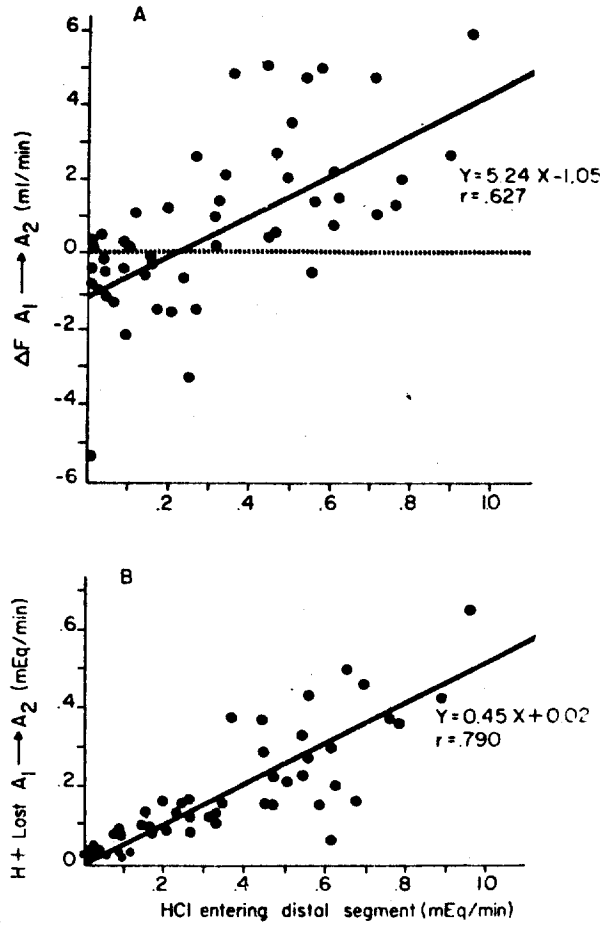

FIG. 6. *A:* net change in flow rate (ΔF) in distal study segme ($A_1 \rightarrow A_2$) related to rate of acid entry into segment. *B:* H^+ loss fr distal study segment ($A_1 \rightarrow A_2$) as a function of rate of acid entry i segment.

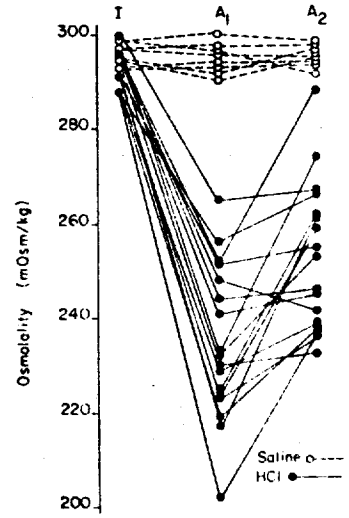

FIG. 7. Osmolality of infused and sampled fluids. I = infus fluid; A_1 = sample aspirated from proximal aspiration tip; A_2 = samp aspirated from distal aspiration tip. In this graph only values o osmolality are shown; no regard here is given to rates of acid or sali infusion (therefore to total solute load).

concentration and in fluid volume at several points in the normal human duodenum. The results indicate that the response to intraduodenal acid instillation is a graded one. Increasing amounts of acid infused into the duodenum evoke increasing amounts of endogenous fluid added to the duodenum. The calculated quantities of fluid added from endogenous sources, while large, are in good agreement with results reported by Demling and Ottenjann (4), Lagerlöf et al. (7), and Wilhelmj and associates (12). Our technique of study differed in several ways from investigations reported by those workers; the major difference was that our controlled but varying rates of acid infusion provided increasing degrees of stimulation of the duodenal mucosa. The results of all these studies, however, demonstrate a marked capacity of the normal human duodenum to dispose of HCl.

The lack of response to the infusion of saline indicates that neither flow nor distention produced by the intraluminal fluid stimulate the outpouring of endogenous fluid. Acid is thus the stimulatory agent. This conclusion is, of course, in accord with the well established observations that acid in the duodenum evokes a flow of pancreatic juice (2), bile (10), and enteric juice (5).

The observed response to acid in the duodenum is likely mediated, at least in part, through the secretin mechanism. It is reasonable to assume that, by increasing the rate of acid infusion, larger areas of duodenal mucosa are bathed by the acid intraluminal fluid, resulting in greater liberation of secretin into the circulation and a greater secretory response to it. The decrease in osmolality observed in the proximal study segment during acid infusions (Fig. 7) was presumably due to the loss of osmotically active particles governed by the chemical equations $HCl + NaHCO_3 \rightarrow NaCl + H_2CO_3$ and $H^+ + HCO_3^- \rightleftarrows H_2CO_3 \rightleftarrows H_2O + CO_2$ (13). In this formulation, the sodium bicarbonate must be added from endogenous sources, and, indeed, the major source would seem to be secretin stimulated, bicarbonate-rich pancreatic juice and bile.

The net gain of fluid over the entire (30-cm) duodenum in response to acid infusion increases as a linear function of the infusion rate, although in less than a unit relationship. The rate of H^+ loss in the duodenum also increases with increasing infusion rates, but in a decreasing increment as the acid infusion rate is increased, suggesting that the process of acid disposal may be a saturable one. In the proximal 15-cm duodenal segment, to which bile and pancreatic juice are added, the rate of fluid addition as well as the rate of acid loss in response to increasing acid infusion rates appear to be limited (Fig. 5, *A* and *B*). The rate of fluid addition appears to reach a maximum at acid infusion rates of 10 ml/min or less, whereas the rate of acid loss appears to do so at acid infusion rates of 1.0 mEq/min or less, further suggesting that the acid disposal process in the proximal duodenal segment is saturable, probably related to limitations of pancreatic and biliary secretion. With higher acid infusion rates, therefore, some of the acid escapes neutralization in the proximal segment and enters the distal segment. Some acid is lost in this segment, presumably without further reaction with bile and pancreatic juice bicarbonate, and is lost regardless of the concomitant loss or gain of fluid in the lumen. The fact that acid disappears from the duodenal lumen coincident with the net absorption of fluid from the lumen (at acid flow rates into the distal segment of up to 0.2 mEq/min) suggests that, either *1*) H^+ is also absorbed, in which case H^+ absorption may be a significant mechanism for H^+ loss from the duodenum, or, alternatively, *2*) bicarbonate enters the lumen (by either diffusion or secretion) and reacts with the H^+ present, in spite of the net absorption of fluid from the segment. The gain in osmolality in the distal segment (Fig. 7) does not favor the latter possibility, since further chemical reaction between HCl and $NaHCO_3$ in the segment should further reduce the osmolality of the luminal fluid. It must be stated, however, that the data of this study do not permit a clear choice between these possibilities.

Wormsley and Brewis (13) have recently questioned the possibility on theoretical grounds that acid in the duodenum can be completely neutralized by $NaHCO_3$ on a mole-for-mole basis, in spite of the fact that most normal human subjects are able to secrete as much bicarbonate into the duodenum as the maximally stimulated stomach is able to secrete acid (1, 14). Preshaw, Cooke, and Grossman (9), on the other hand, showed that in the dog the amount of pancreatic bicarbonate secreted in much less than enough to neutralize the amount of acid required in the duodenum to elicit that response. Whether this situation pertains to the human is not known.

We thank Mrs. Margaret Gasiorowski for invaluable help with the studies, Mrs. Monika Minuth and Mrs. Jacqueline Terwilliger for expert assistance with the manuscript, and Dr. Konrad H. Soergel for helpful criticism and encouragement through all phases of the work.

This work was supported in part by Grants 5-M01-FR-00058, RR-00287-05, and AM-14773-01 from the National Institutes of Health and a grant from the August and Emily Werdehoff Uihlein Fund.

Received for publication 19 August 1971.

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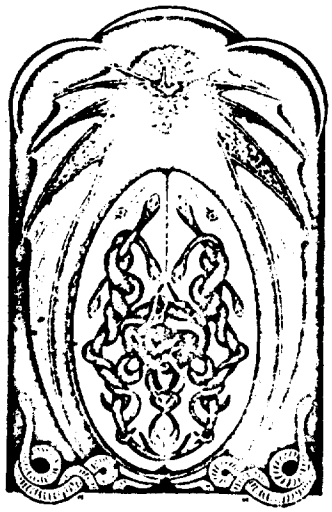
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