cell types to the labeled population.

Age, sex, and hormonal state are some of the factors that influence the numbers of anterior pituitary nuclei in DNA synthesis. Female rats had higher labeling indices than males and young females showed more frequent pituitary labels than adult females with their growth phase completed. Cortisone treatment of adult males produced a 6-fold depression in the labeling index of the anterior pituitary. Previous studies with cortisone or hydrocortisone have shown a similar reduction in the numbers of DNA-synthesizing nuclei in a variety of tissues including the zona fasciculata of the adrenal, articular and growth-plate cartilate(6) and various cells of the cardiovascular-renal system(2). An exception is the pancreas, for when steroid diabetes is produced the islet tissue increases in bulk and thymidine labeled nuclei in islet cells become more numerous(1).

DOC has no comparable effect on pituitary labeling but when it is combined with an increased dietary load of sodium chloride in unilaterally nephrectomized rats and hypertension is produced there is a significant increase in the pituitary labeling index. This is coupled with an increase in the numbers of DNA-synthesizing nuclei in the hypertensive damage that occurs in the heart, arteries, and kidneys of such rats(2). It is not clear that this alteration in pituitary cell turnover represents a response to the increased blood pressure, for when a severe degree of hypertension was established by the adrenal regeneration procedure of Skelton(5) the general level of pituitary labeling is decreased as compared with control animals. A comparison of the effect of hypertension in these circumstances is not necessarily valid since the age and sex of the 2 groups of hypertensive rats differed, and these factors and the stage of the oestrus cycle seem to have important effects on the magnitude of cell turnover in the anterior pituitary.

Summary. The numbers of nuclei in the DNA synthetic phase of the nuclear cycle were estimated in the rat anterior pituitary by ^{3}H-thymidine autoradiography. Age, sex, and hormonal state produced significant shifts in the labeling frequency. Radioactive nuclei were more numerous in young females and less frequent in adult males. The low labeling frequency in adult male rat pituitary was further depressed by cortisone administration.

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1. Crane, W. A. J., Dutta, L. P., J. Endocrin., 1964, v28, 341.

2. Crane, W. A. J., Ingle, D. J., Arch. Path., 1964a, v78, 209.

3. ———, ibid., 1964b, in press.

4. Selye, H., Hall, C. E., Rowley, E. M., Canad. Med. Assn. J., 1943, v49, 88.

5. Skelton, F. R., Proc. Soc. Exp. Biol. and Med., 1955, v90, 342.

6. Young, M. H., Crane, W. A. J., Ann. Rheum. Dis., 1964, v23, 163.

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Effect of Alternating Acid and Alkaline Digestive Fluids on the Gastrointestinal Tract of the Rat.* (30128)

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The formation of gastric and duodenal ulcer in man is usually attributed to effects of acid-pepsin on susceptible areas. A few authors have suggested that tryptic origin of ulcer may be possible(1-3). In earlier experiments one of us has found that a frog's leg exposed for a short period of time to acid or

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acid-pepsin was not digested; however, when this was followed by exposure to alkaline trypsin solution, digestion occurred(4-6). We have considered therefore the possibility that in the duodenal bulb alternation between alkaline pancreatic and acid gastric juice may play a role in the production or chronicity of duodenal ulcer.

Methods. A total of 77 adult albino rats of the Holtzman strain, 43 male and 34 female, with body weights between 178 to 400 g and an average weight of 276 g, were fasted for 48 hours, with water available. They were anesthetized with 0.06 to 0.08 ml of 6% nembutal solution per 100 g of body weight given intraperitoneally. To keep the airways open, a small catheter was placed and secured in the upper trachea. The pyloric sphincter was kept open by insertion of a short piece of polyethylene tube and tied in place, avoiding ligation of blood vessels.

In most experiments the esophagus was cannulated and perfused fluid traversed the entire gastrointestinal tract, leaving through a tube in the terminal ileum. In other experiments, the upper esophagus was cannulated and the fluid traversed esophagus, stomach and duodenum. In a smaller group of experiments, the upper duodenum was intubated and the fluid traversed the entire small intestine, leaving at the lower ileum. In all experiments, the common bile duct was cannulated at the duodenum and emptied to the outside; consequently, bile and pancreatic secretions were eliminated. Fluids were perfused at constant rates of 0.8 ml per minute at 38°C. The fluids were HCl, pH 1.4, and $NaHCO_3$, pH 8, without or with pepsin Armour 0.5%, or pancreatin Wilson 1% respectively. When solutions alternated every 15 minutes, the perfused segments were washed out with 5 ml of 0.9% saline solution. Immediately after termination of each experiment, the entire gastrointestinal tract was opened, washed carefully with saline and examined under a binocular microscope.

Pepsin Armour, 0.2 mg, incubated for 10 minutes at 37°C with Anson's hemoglobin method yielded 0.4576 mg of Tyrosin. Trypsin in Pancreatin Wilson, assayed by a similar method, yielded 0.4272 mg of Tyrosine per 0.2 mg of Pancreatin.

TABLE I. Grades of Lesions in Gastrointestinal Tract.

Grade	State of gastrointestinal wall
0	Normal.
1	Edematous and/or hyperemic mucosa.
2	White-cloudy and necrotic mucosa, usually with scattered small bleeding points.
3	Erosion of epithelium and ulceration into submucosa.
4	Digestion into muscular layers, transparency of entire wall, serosal vessels seen clearly from mucosal side.

Gradations of lesions in the gastrointestinal tract are presented in Table I. Data on animals that died during an experiment are excluded. Likewise, changes in the intestine within 1 cm from ligatures are not included, because circulation in this area suffered and lesions were due to this interference and not to the perfusion.

In 13 experiments, changes in blood pH were measured every 15 minutes during perfusion of acid pepsin and/or alkaline pancreatin for 90 minutes with a micro pH meter (Astrup). To 6 of these animals 2 × 5 ml of 0.5%, 2%, or 4% $NaHCO_3$ solution was administered intraperitoneally during perfusion, in order to counteract acidosis due to acid perfusion.

Results. Table II. Our results with perfusion from esophagus to terminal ileum for 90 minutes with alkali-pancreatin (Group 1) demonstrated very little damage to the mucosa, grade 0-1. On the other hand, in Group 2 perfusion with acid-pepsin for 90 minutes produced more serious damage, grade 1-2. In Group 3, where acid-pepsin and alkaline pancreatin of the same concentration as in Groups 1 and 2 alternated every 15 minutes for 90 minutes, severe damage of grade 3 to 4 occurred in the duodenum, jejunum, and ileum. It is significant that in Group 3 acid-pepsin acted only for a total of 45 minutes. Alternating perfusion with acid and bicarbonate (Group 4) or with HCL-pepsin and alkali (Group 5) or with HCL and alkali pancreatin (Group 6) produced some damage but not as severe as in Group 3.

TABLE II. Results of Perfusion from Upper Esophagus to Lower Ileum in Rats.

Group No. and procedures	Total No. of rats	Esophagus		Prostomach		Glandular stomach		Duodenum		Jejunum		Ileum	
		No. of rats	Grades of lesions	No. of rats	Grades of lesions	No. of rats	Grades of lesions	No. of rats	Grades of lesions	No. of rats	Grades of lesions	No. of rats	Grades of lesions
1. Alkaline pancreatin	5	1	0	1	0	1	0–1	2	0–1	5	1	1	0–1
		2	0–1	1	0–(1)	4	1	3	1			2	(0)–1
		2	1	2	0–1							2	1
				1	(0)–1								
Average			0–1		0–1		1		(0)–1		1		(0)–1
2. Acid pepsin	9	6	1	5	1	2	1–(2)	8	2	6	2	1	(1)–2
		1	1–(2)	3	1–(2)	6	1–2	1	2–(3)	3	2–(3)	8	1–2
		2	1–2	1	1–2	1	2						
Average			1–(2)		1–(2)		1–2		2		2		1–2
3. Alternating 15′ Acid pepsin and alkali pancreatin	21	5	0–1	3	0	9	1	1	2	1	(1)–2	2	2
		8	1	10	0–1	2	1–(2)	1	2–(3)	1	2–3	1	2–(3)
		3	1–(2)	6	1	3	1–2	3	2–3	2	3	6	2–3
		1	1–2	1	1–2	5	2	2	(2)–3	3	3–(4)	1	(2)–3
		4	2	1	2	1	2–3	8	3	6	3–4	5	3
						1	3	1	3–(4)	6	(3)–4	4	3–(4)
								5	3–4	2	4	1	3–4
												1	(3)–4
Average			1		(0)–1		1–2		3		3–4		(2)–3
4. Alternating 15′ HCl and $NaHCO_3$	6	4	0–1	2	0	5	1	1	1–2	1	1–2	2	1
		2	1	4	0–1	1	1–(2)	1	(1)–2	1	2	3	1–(2)
								3	2	3	2–(3)	1	1–2
								1	2–(3)	1	2–3		
Average			(0)–1		0–(1)		1		2		2		1–(2)
5. Alternating 15′ Acid pepsin and $NaHCO_3$	6	4	0–1	2	0	6	1	4	2	1	2	1	1
		2	1	4	0–1			2	2–3	4	2–(3)	1	1–(2)
										1	2–3	1	(1)–2
												1	2
												1	2–(3)
												1	2–3
Average			(0)–1		0–(1)		1		2		2–(3)		(1)–2
6. Alternating 15′ HCl and alkali pancreatin	6	3	0–1	4	0–1	6	1	5	2	1	2	6	2
		3	1	2	1			1	2–(3)	4	2–(3)		
										1	2–3		
Average			(0)–1		(0)–1		1		2		2–(3)		2

Perfusion rate, 0.8 ml/min; temp, 38°C; total perfusion time, 90 min.

In Group 2, much hemorrhage, often diffuse, particularly in the mucosa of duodenum, jejunum and ileum was seen. Group 1 had no hemorrhage, while Groups 3-5 had pinpoint hemorrhages, most in duodenum and jejunum.

Table III. Alternating perfusion from esophagus to duodenum for 90 minutes showed relatively little damage in esophagus and stomach, but severe damage in duodenum. Alternating perfusion from duodenum to ileum for 90 minutes was followed by severe damage to all segments, often with pinpoint hemorrhages. These results were similar to those in Group 3 in Table II.

Fig. 1. The solutions used, mainly the acid

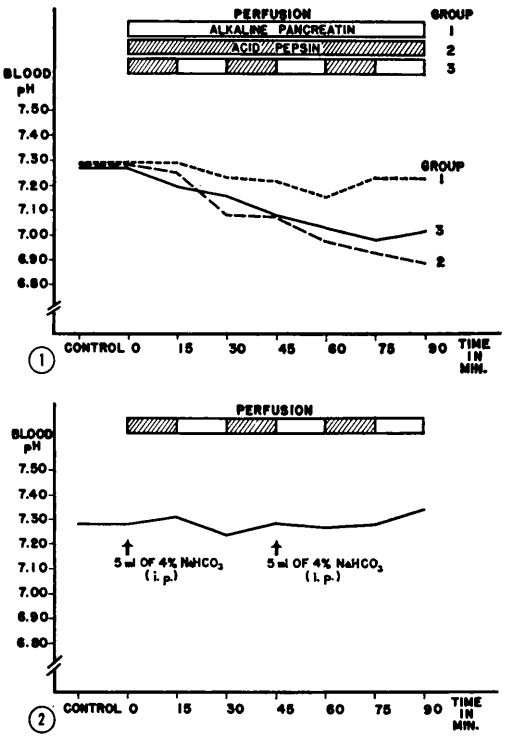

FIG. 1. Effect of perfusions on blood pH of rats, 90 min perfusion from esophagus to terminal ileum, infusion rate 0.8 ml/min, temp 38°C. Group 1. Perfusion with alkaline pancreatin solution (white bar). Group 2. Perfusion with acid pepsin (hatched bar). Group 3. Perfusion with alkaline pancreatin and acid pepsin solution, alternating every 15 min.

FIG. 2. Effect on blood pH of i.p. injection of 2×5 ml of 4% $NaHCO_3$ during perfusion from esophagus to terminal ileum with acid pepsin and alkaline pancreatin for 90 min, alternating every 15 min. Infusion rate 0.8 ml/min at 38°C.

TABLE III. Alternating Perfusion with Acid Pepsin and Alkaline Pancreatin from Esophagus to Duodenum and from Duodenum to Terminal Ileum in Rats.

Procedures	Total No. of rats	Esophagus No. of rats	Esophagus Grades of lesions	Prostomach No. of rats	Prostomach Grades of lesions	Glandular stomach No. of rats	Glandular stomach Grades of lesions	Duodenum No. of rats	Duodenum Grades of lesions	Jejunum No. of rats	Jejunum Grades of lesions	Ileum No. of rats	Ileum Grades of lesions
Alternating 15' Esophagus	8	4	0	2	0	5	1	3	2				
Acid pepsin and to duodenum		2	1	2	1	3	2	2	3				
alkali pancrea-		2	2	3	2			3	4				
tin				1	3								
Avg			(0)–1		1–(2)		1–(2)		3				
Duodenum to ileum	3							2	(2)–3	1	3–(4)	1	3
								1	3	2	3–4	1	3–(4)
												1	3–4
Avg									(2)–3		3–4		3–(4)

Perfusion rate, 0.8 ml/min; temp, 38°C; total perfusion time, 90 min.

ones, affected blood pH. In Group 1, perfused with alkaline enzyme fluid, a slight acidosis was seen. In Group 2 with acid-pepsin and in Group 3 with alternating acid and alkali enzyme perfusion, a considerable degree of acidosis occurred.

Fig. 2. In order to check whether acidosis might contribute to development of lesions produced by alternating acid-alkali enzyme perfusion, the animals received i.p. injections of sodium bicarbonate which kept blood pH at or near control levels. Ten ml of a 4% solution of sodium bicarbonate was found more adequate for this purpose than 10 ml of 0.5 or 2% solutions. However, adjustment of the pH during perfusion in rats with alternating acid and alkaline enzyme solutions did not change the deleterious effects when compared with rats with acidosis (Table I).

Discussion and conclusions. Alternating 15-minute perfusion of duodenum and jejunum with acid-pepsin for a total of 45 minutes and with alkaline pancreatin solution for 45 minutes had more marked effects than perfusion with acid pepsin alone for 90 minutes. Surprisingly, esophagus and stomach of the rat were more resistant to HCL-pepsin perfusion than described in other animals (6). Also, surprisingly, no ulcers were formed in stomach, duodenum, jejunum and ileum, but necrosis, mucosal digestion and hemorrhages were frequent. Changes of blood pH did not seem to change the results of alternating perfusion with acid-alkaline enzyme solutions. Similar observations have been reported on the bullfrog(7).

In 3 rabbits and in 1 cat, perfusion of the gastrointestinal tract with alternating acid-pepsin and alkaline pancreatin solutions for 60 to 120 minutes yielded results almost similar to those described above in the rat; in another cat, perfusion with acid-pepsin solution for 120 minutes likewise produced similar results as in the rat, except for hemorrhagic lesions in the lower esophagus.

We believe that our experiments demonstrate that alternation between acid-pepsin and alkaline pancreatin can produce more damage to the small intestine of the rat than acid-pepsin alone. This raises the question again whether such mechanism may play a role in ulcer disease in man.

We believe that HCL-pepsin in some way prepares superficial layers of tissues and thereby permits subsequent digestion by alkaline trypsin. There is the possibility therefore, that alternation between gastric acid-pepsin and alkaline pancreatic secretions in the duodenum can contribute to the development or the chronicity of duodenal ulcer. However, other factors, such as inadequate blood supply, focal weakness of the mucosa, etc., must be present initially.

Summary. Previous experiments have demonstrated that exposure of a frog's leg alternately to acid-pepsin and alkaline pancreatic secretion produced rapid digestion of the leg. It was suggested that in man the rapid interchange between acid pepsin and alkaline trypsin solution in the duodenal bulb might play a role in peptic ulcer. For this reason, 5 kinds of perfusion from esophagus to ileum were conducted in rats. Alternating perfusion with acid pepsin and alkaline pancreatin (Group 3) had more marked effects in duodenum, jejunum, and ileum, than perfusion with acid pepsin alone (Group 2), or other perfusions (Group 1, 4, 5), for 90 minutes. Similar results were observed on cats and rabbits. The esophagus of the rat seemed to be more resistant to such perfusions than that of the cat. Changes of blood pH due to alternating perfusion with acid-alkaline enzyme solutions did not seem to affect the deleterious effects. While these results are suggestive of a role of alternation between gastric and pancreatic juice in ulcer disease, no conclusions concerning development of peptic ulcer can be drawn.

1. Boldyreff, W., Arch f.d. ges. Physiol., 1907-8, v121, 13.

2. Kirchheim, L., Böttner, A., Arch. exp. Path. u. Pharm., 1915, v78, 99.

3. Stuber, B., Z. F. exp. Path. u. Ther., 1914, v16, 295.

4. Necheles, H., Levitsky, P., Maskin, M., Proc. Soc. Exp. Biol. and Med., 1936, v34, 768.

5. Callahan, R., Maskin, M., Necheles, H., Am. J. Dig. Dis. & Nutr., 1936, v3, 174.

6. Perry, J. F., Jr., Yonehiro, E. G., Ya, P. M., Root, H. D., Wangensteen, O. H., Proc. Soc. Exp.

Biol. & Med., 1956, v92, 237.

7. Necheles, H., Neuwelt, F., Am. J. Dig. Dis., 1937, v4, 453.

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Effect of 1-Methyl-2-Mercaptomidazole (Methimazole-Tapazole) on Feed Consumption in the Rat.* (30129)

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In the estimation of the thyroid hormone secretion rate (TSR) in rats in our laboratory(1), a goitrogen, methimazole or tapazole, has been used to block the recycling of I^{131} resulting from the metabolism of thyroidal-I^{131}. By blocking the uptake of I^{131} by the thyroid gland, the apparent release rate of thyroidal-I^{131} is increased and the end-point at which thyroidal-I^{131} is blocked by increaseing levels of thyroxine (T_4) is more readily determined.

While the goitrogen, propylthiouracil, has been shown to accelerate the rate of thyroidal-I^{131} release(2), methimazole was apparently without extra thyroidal effects in the rat(3).

The present study was initiated to determine the effect of methimazole on feed consumption of the rat. If methimazole seriously depressed consumption, it would constitute an objection to its use, since decreased feed consumption depresses TSR of rats(4). In previous work, it has been shown that thyroparathyroidectomy depressed food intake 13% in a period of 10 to 16 days(6).

Materials and methods. Eighteen female Sprague-Dawley Rolfsmeyer rats with a mean body weight of 215 g were placed in metabolism cages constructed so as to prevent feed loss and coprophagy. Temperature was maintained at 78 ± 1°F and artificial illumination was provided during normal daylight hours. Purina Lab Chow with an energy value of 4.41 calories per g and 23.4% total protein was provided daily and resulting residues were weighed back. Mean daily feed consumption was determined for a period of 10 days, followed by the mean feed consumption at 10-day intervals for 30 days under the influence of methimazole.

For normal controls, a second group of 18 rats weighing 231 g were fed for 30 days. Their mean daily feed consumption was determined at 10-day intervals to compare with the experimental group. The level of methimazole which has been used to prevent the recycling of I^{131} in TSR studies has been 400 μg/100 g bw/day by subcutaneous injection. Prior to the 10-day control feeding period, I^{131} was injected into each rat to determine normal uptake of I^{131} by the thyroid gland measured externally by a scintillation detector. After 2 weeks, and again after 4 weeks of methimazole feeding I^{131} was again injected to determine the uptake of I^{131}. Four days after methimazole was withdrawn and again after 7 days, I^{131} was injected to determine the % uptake.

The mean pituitary and thyroid gland weights of a control group of 10 rats was compared with a group of 10 rats at the end of the 30-day methimazole feeding experiment and of a group of 8 rats 7 days after methimazole withdrawal.

Results. The mean feed consumption of the 18 normal control rats weighing 231 g during the first 10-day period was 13.35 g/day or 5.78 g/100 g bw/day. During the second 10-day period these rats consumed 13.34 g/day or 5.63 g/100 g bw/day, a reduction of 3%. During the final 10-day period feed consumption was 13.73 g/day and 5.70 g/100 g bw, a decrease of 1% (Table I). Since the feed consumption of the control group during the 30-day period was essentially constant, the effect of methimazole on

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