0.2 mg of dialyzate kg to produce a 2-fold elevation of plasma cholesterol.

Discussion. Our previous work established that the pituitary was necessary for release of LM. The data presented in this paper establish that hog posterior pituitary dialyzate contained LM activity comparable to that of plasma dialyzate. Other points of similarity between plasma LM and posterior pituitary LM were observed in groups of 10 rats administered 1 mg/dialyzate kg. The hyperlipemia occurring in either group 45 minutes later was not abolished by administration of 400 U of heparin kg. *In vitro* anti-clearing activity of pituitary LM was identical with the activity previously reported for plasma LM(1,2). Exact identity of pituitary LM with plasma LM remains to be established.

Summary and conclusions. 1. Dialyzate of hog posterior pituitary lobe induced hyperlipemia in rats, mice, guinea pigs, dogs and rabbits. 2. The LM activity was indistinguishable from that of plasma dialyzate from cortisonized animals. 3. Neither Pitressin® nor Pitocin® had LM activity. 4. Hog anterior pituitary extracts had no LM activity. 5. LM appears to be a hitherto undescribed hormone either elaborated or stored in the posterior pituitary.

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Inhibition of Histamine-Stimulated Secretion by Topical Application of Antihistaminics to the Canine Mucosa.* (23207)

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Failure of antihistaminics to inhibit histamine-stimulated gastric acid secretion is generally accepted. The extensive literature supporting this generalization has been carefully summarized by Robertson and Grossman(1). The gastric receptors for histamine, therefore, appear to present a puzzling exception to the almost universal blocking action of antihistaminics on histamine receptors in other organs. These authors, however, also have collected some fragments of evidence which indicate that high concentrations of antihistaminic compounds, present locally in the gastric mucosa, may block its secretory response to histamine. Wood(2) was able to block histamine-stimulated acid secretion in anesthetized cats by retrograde injection of phenergan, neoantergan, and antistin into the ligated splenic artery, so that these compounds reached the gastric mucosa in high concentration. A chemical analogue of phenergan without antihistaminic activity (R.P. 3300) was without effect. In their own experiments, Robertson and Grossman irrigated canine pouches with high concentrations of several antihistaminics (25 and 50 mg/ml of neoantergan; 6.25, 12.5, and 25 mg/ml of pyribenzamine; 12.5 and 25 mg/ml of benadryl; and 50 mg/ml each of trimeton and chlortrimeton) and thereby inhibited the secretion of acid stimulated by histamine. However, these authors believed that, strictly speaking, their results were not "antihistaminic" but rather a direct irritant effect on the gastric glands, since 1) these compounds inhibited acid secretion induced by the parasympathomimetic drug, urecholine, as well; and 2) these compounds stimulated flow of a mucoid liquid from the pouches, which was frequently bloody. The concentrations of drug which

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TABLE I. Effect of Antihistaminics, Applied Topically to Gastric Mucosa of Canine Heidenhain Pouches, on Histamine-Stimulated Gastric Acid Secretion.

Compound	Conc. (mg/ml)	Output of HCl (mEq) Control	Test	% decrease	% inhibition (mean % decrease)
Isotonic saline		2.23	1.94	13	
		1.17	1.11	5	
		2.26	2.26	0	
		1.94	.69	13	
		1.96	2.15	10	
		4.10	2.58	37	
		1.40	1.57	12	
		1.50	1.47	2	
		4.16	4.23	2	
		3.09	3.30	7	10
Phenergan hydrochloride	2	2.45	.84	66	
		1.04	.08	92	
		3.26	.47	86	
		3.39	2.41	29	
		3.97	.73	82	
		2.52	.31	88	
		.27	.00	100	
		.57	.04	93	
		1.79	.62	65	
		1.55	.45	71	
		.30	.00	100	80
	4	.58	.00	100	
		.52	.00	100	
		.97	.00	100	
		3.03	.00	100	
		2.08	.00	100	
		1.48	.00	100	100
Phenergan methosulfate	2	.60	.43	28	
		2.18	1.02	53	
		.91	.42	54	
		.55	.40	27	41
	4	1.97	.71	64	
		1.43	.70	51	
		1.62	.88	46	
		.88	.40	55	
		.63	.33	48	
		2.88	1.22	58	54
Pyribenzamine	2	3.25	2.67	18	
		3.18	1.18	63	41
	4	2.76	1.98	28	
		3.38	1.45	57	
		2.75	1.54	44	
		.72	.24	67	
		1.75	.16	91	57
	6	1.48	.26	82	
		.49	.11	79	
		2.12	.83	61	74
	8	2.02	.72	64	
		2.10	.34	84	
		.61	.08	87	
		1.78	.50	72	77
Neoantergan	8	.75	.19	75	
		.36	.19	47	61
	10	1.99	.77	61	
		1.28	.40	69	65

TABLE II. Effect of Antihistaminics, Applied Topically to Gastric Mucosa of Canine Heidenhain Pouches, on Urecholine-Stimulated Gastric Acid Secretion.

Compound	Conc. (mg/ml)	Output of HCl (mEq) Control	Test	% decrease	% inhibition (mean % decrease)
Phenergan hydrochloride	2	.21	.00	100	
		2.36	.76	68	
		1.09	.64	41	
		.66	.00	100	77
	4	.87	.10	89	
		.91	.00	100	
		.20	.00	100	
		.52	.00	100	97
Phenergan methosulfate	2	.10	.05	50	
		.04	.02	50	50
	4	.45	.14	69	
		.73	.09	88	79
Pyribenzamine	6	.31	.04	87	
		.13	.00	100	
		.23	.00	100	
		.56	.22	61	87

they employed caused convulsion in one animal and death in another.

The present experiments, begun independently of the foregoing, demonstrate the inhibition of histamine-stimulated gastric acid secretion when considerably lower concentrations of antihistaminics are applied to the mucosa of canine pouches. However, topical application of a surface-active local anesthetic, nupercaine, also inhibits such secretion in comparable degree, and this limits definitive interpretation of these experiments, at least for the time being.

Methods. In this study vagally denervated corpus pouches were used, and the response to histamine (0.1 mg base kg body weight), given subcutaneously, was measured before and after application of one of several antihistaminics. The inhibitor was instilled into the pouch for a total of 30 minutes, the solution being exchanged for a fresh one at the end of the first 15 minutes. Concentrations of the drug in isotonic sodium chloride ranged from 2 to 10 mg/ml, and volumes of 25-50 ml were required to distend the pouches slightly with hand pressure on the syringe plunger. The antihistaminic compounds used were phenergan, phenergan methosulphate, pyribenzamine, and neoantergan; the local anesthetic agent was nupercaine. In a second series of experiments, gastric secretion was stimulated by the vagomimetic drug, urecholine (carbamyl methylcholine chloride), administered subcutaneously at a dosage of 0.1 mg/kg body weight.

Results. Although the gastric acid secretory response to a fixed dose of histamine may vary considerably from day to day in the same animal, the response to 2 successive doses given to the same animal on the same day (as in the so-called "tandem histamine test") show more regularity. To confirm this, and to control any effect of the application of isotonic saline itself, 10 experiments were performed in which 2 doses of histamine were given at an interval of 2¼ hours, with an application of saline to the pouch for 30 minutes between doses. From Table I, it will be seen that the percentage difference between the first (control) and second (test) responses in output of HCl varied from 0 to 37, with a mean of 10%. In all subsequent experiments using a drug, the doses of histamine were given at the same interval.

The effect of local application of several antihistaminics (phenergan, phenergan methosulphate, pyribenzamine, and neoantergan) on output of histamine-stimulated acid from pouch dogs is also shown in Table I. A significant degree of inhibition of acid output occurred at each dose level with each of these compounds, being greatest with phenergan at a concentration of 4 mg/ml (100% in-

TABLE III. Effect of a Topical Anesthetic (Nupercaine), Applied to Gastric Mucosa of Canine Heidenhain Pouches, on Histamine- and Urecholine-Stimulated Gastric Acid Secretion.

Stimulus	Nupercaine conc. (mg/ml)	Output of HCl (mEq) Control	Test	% decrease	% inhibition (mean % decrease)
Histamine (.1 mg/kg)	1	.79	.82	4	
		2.22	1.53	31	18
	2	2.13	1.40	34	
		3.55	1.34	62	
		2.60	1.35	48	
		3.52	3.07	13	
		.48	.24	50	
		1.34	.87	35	
		2.14	1.62	24	
		.83	.54	35	38
	4	.40	.12	70	
		.75	.17	77	
		.80	.00	100	
		2.25	.98	56	76
Urecholine (.1 mg/kg)	2	.68	.91	0	
		.48	.49	0	
		.61	.57	7	2
	4	.10	.00	100	
		.37	.00	100	100

hibition).

The results of experiments in which urecholine (0.1 mg/kg) was used to stimulate gastric acid secretion are shown in Table II. With this stimulus also, antihistaminic phenergan effected a virtually complete inhibition at a concentration of 4 mg/ml, and significant inhibition was elicited by phenergan methosulphate, and pyribenzamine at concentrations of 4 or 6 mg/ml.

Of considerable interest is the fact that inhibition could likewise be elicited with a representative topical anesthetic. Thus, nupercaine at a concentration of 4 mg/ml induced a mean degree of inhibition of 76% in a number of experiments with histamine stimulation, and 100% in those in which urecholine was the stimulus (Table III).

The topical application of these agents to the pouch mucosa was well tolerated systemically by these animals, even at the low concentrations used. Following instillation of phenergan at 4 mg/ml, there occurred a significant flow of a non-acid mucoid fluid, smaller in volume but similar in appearance to that which has been obtained in a similar investigation in this laboratory, following instillation of the enzyme-inhibitors iodoacetamide and N-ethyl maleimide(3). This happened in both the histamine and the urecholine experiments. None of the other inhibitors gave rise to this mucoid fluid except in several of the experiments with nupercaine in which HCl secretion was prevented entirely. Duration of drainage of this non-acid fluid never lasted longer than 3 hours, and usually only about 2, in contrast with 8 hours or more after the enzyme-inhibitors used in the previous investigation. The specimens were blood-tinged (never frankly bloody) in a few of the experiments, which is believed to have resulted from slight mechanical trauma by manipulation of the catheter during the instillation.

Discussion. The present experiments demonstrate that application of several histamine-blocking agents locally to the gastric mucosa of vagally denervated pouch dogs can inhibit secretory response to histamine in considerable degree. Thus, they confirm the findings of Wood in the cat(2), and of Robertson and Grossman in the dog(1). In contrast to the latter authors' report, however, the concentrations of drug used herein were low, no frankly bloody mucus was produced, and no untoward systemic effects of the compounds were elicited. The inhibitory effect of antihistaminic drugs on secretion induced vago-

mimetically was demonstrated by blocking urecholine-stimulated secretion with several of these agents, thus confirming the report of Robertson and Grossman on pyribenzamine. The finding that a local anesthetic agent can inhibit both histamine-stimulated and urecholine-stimulated acid secretion is also of great interest. In all these experiments, the mean degree of inhibition is clearly related to the dosage employed, and actually reaches 100% in the phenergan-histamine and nupercaine-urecholine experiments.

Robertson and Grossman were reluctant to ascribe any specific significance to the results of their experiments on 2 grounds. First, because of the production of gastric irritation and bloody mucous secretion from the pouch, they ascribed a non-specific role to the antihistaminics employed. Our experiments meet this objection since concentrations of drug used were low, no local irritant effects were produced, and no systemic symptoms were elicited. Some non-acid mucoid fluid was produced with phenergan and nupercaine in those experiments in which acid inhibition was complete. However, the similarity of this material to the mucous secretion obtained after inhibition with N-ethyl maleimide and iodoacetamide, and the fact that the latter is unequivocally differentiated from interstitial fluid or transudate by its content of nitrogen, hexosamine, and reducing substances(3), make it likely that this is some secretory rather than "irritant" inflammatory product. Secondly, inhibition of the response to a parasympathetic stimulant (urecholine) as well as to histamine was interpreted by Robertson and Grossman as evidence definitely against specific antihistaminic action of the drugs. This is not crucial to the argument for specificity, since these same authors have previously shown(4) that hog renal cortex histaminase inhibits urecholine-stimulated gastric secretion, as well as that induced by histamine and food, and it may be argued that a cholinergic mechanism is intermediate in the histamine effect, or vice versa. The recent demonstration in this laboratory(5) of inhibition of histamine acid secretion by atropine is consistent with such an interpretation. On the other hand, the inhibition of histamine-stimulated secretion by the local anesthetic, nupercaine, indicates that there are limitations to interpreting the antihistaminic effect as a specific one, especially since the antihistaminic compounds are also known to be local anesthetics.

Kay and Forrest(6) have also recently reported that the antihistaminic promethazine and mepyramine, when applied locally to the mucosa of Heidenhain pouches, will inhibit the acid secretory response to histamine. They have argued that this is a specific effect rather than a local anesthetic one because, although these two compounds have both local anesthetic and antihistaminic properties, promethazine was more potent than mepyramine in its antihistaminic and its acid inhibitor activity. This argument, however, is of limited force, because these authors did not directly study the effects of local anesthetics, and even more so because the present study indicates that local anesthetics can inhibit histamine- and vagomimetic-stimulated acid secretion when the concentrations of drug are large enough.

Current evidence indicates that there are at least 3 possible modes of action of the antihistaminics in blocking histamine-stimulated acid secretion: (1) a specific antihistaminic effect, (2) the well known atropine effect of these compounds, and (3) their local anesthetic action. In addition to these well-established properties, it is conceivable also that these substances may act by way of some effect on intracellular processes of acid formation. The present experiments do not allow of any definitive choice among these possibilities. Thus, the failure to achieve antihistaminic blocking effects on the stomach by the parenteral administration of antihistaminic compounds is still puzzling, and from the therapeutic point of view an extremely disappointing phenomenon.

Summary and conclusions. 1. Topical application of several antihistaminic drugs to the mucosa of canine corpus pouches significantly inhibited the acid secretory responses of these pouches to parenterally administered histamine. One local anesthetic agent elicited similar inhibition. These inhibitory substances were effective also against a vagomi-

metic stimulant, urecholine. 2. In an effort to explain these phenomena, 3 possible modes of action of the antihistaminics present themselves: (1) a specific (antihistaminic) effect, (2) an atropine effect, (3) a local anesthetic effect. The evidence presently available does not as yet permit of any preference among these several hypotheses.

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Efficiency of Intestinal Absorption of Vitamin B_{12} Measured by Hepatic Uptake of $Co^{60}B_{12}$.* (23208)

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In an earlier work(1) we studied the intestinal absorption of vit. B_{12} by means of measurement of hepatic uptake of $Co^{60}B_{12}$ (2). Booth and Mollin had compared this method with measurement of fecal excretion of $Co^{60}B_{12}$ and showed that hepatic uptake of radioactive B_{12} a week after oral administration is proportional to amount of B_{12} absorbed in the intestine(3). We have also expressed the efficiency of intestinal absorption of vit. B_{12} in terms of parenteral equivalents (1), by repeating measurement of hepatic uptake in the same individual after oral and parenteral administration of similar doses of $Co^{60}B_{12}$. Pollycove and Apt(4) demonstrated that the determination of efficiency of intestinal absorption of B_{12} by this method gives results similar to those obtained by measurement of fecal excretion of $Co^{60}B_{12}$. The present investigation was undertaken to add more information on the efficiency of intestinal absorption of vit. B_{12} under various pathological conditions, by this technic.

Method. Hepatic uptake of $Co^{60}B_{12}$ was measured by a previously described method (2) on 31 patients with a variety of diseases, and a total of 112 individual tests were made. The determinations were done 6-7 days following a) oral administration of tracer dose of 0.5 μg $Co^{60}B_{12}$ containing 0.35 resp. 0.50 μc Co^{60}†, b) same dose of $Co^{60}B_{12}$ together with potent intrinsic factor preparation‡, c) same dose of $Co^{60}B_{12}$ intramuscularly. Each of these 3 tests was done on the same patient at intervals of 10-14 days. This is the time interval at which a plateau of hepatic counts appears(2-6). This leveling off of radioactivity over the liver makes it possible to use final radioactivity counts in one test as a base line for the next. As many as 4-6 individual tests were done on 12 patients. Efficiency of intestinal absorption of B_{12} was calculated in terms of parenteral equivalents according to the formula:

$$\text{Parenteral equivalent of intestinal absorption (in \%)} = \frac{\text{Hepatic uptake on oral admin.}}{\text{Hepatic uptake on parenteral admin.}} \times 100.$$

The cases included 7 patients with pernicious anemia, 4 with sprue, 3 with macrocytic

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‡ Supplied through the courtesy of Dr. K. W. Thompson of Organon, Inc., Orange, N. J. The preparation used (#5333) did not contain any appreciable amount of B_{12} on microbiological assay and was used at dose of 62 mg, equivalent to dose contained in 2 USP units of oral anti-anemia preparation.