TABLE I. Comparison of Calcium Concentration in Plasma and Tissue Between Control and Parathyroidectomized Male Rats.

	Calcium as mg/100 g wet wt of tissue*			
	Plasma	Submandibular salivary gland	Muscle	Kidney
Sham operated control	10.1 ± .07	27.1 ± 1.30	6.37 ± .06	6.41 ± .06
Parathyroidectomized	6.80 ± .16	36.5 ± 1.10	5.76 ± .04	5.65 ± .04

* Values reported as arithmetic mean ± standard error for groups of 14 rats.

cium concentration on the submandibular gland in the absence of parathormone may reflect an increased transport of calcium into the gland and subsequent binding with glycoprotein. Parathyroidectomized lactating rats have an increased calcium concentration in the milk. It has been suggested that the increased calcium concentration of the milk from parathyroidectomized lactating rats is due to an increase in the transport of calcium to the mammary gland in the absence of parathormone(9).

Summary. Submandibular salivary glands of parathyroidectomized rats have an increased calcium content compared to control rats. Kidney and muscle from parathyroidectomized rats had decreased calcium concentrations paralleling the plasma calcium changes.

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Gastric Inhibitors in Fasting Canine Thoracic Duct Lymph.* (30462)

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Gastric juice, especially that from the antrum, has been found to contain a substance (G.I.S.) which inhibits gastric secretion upon intravenous injection in dogs or rats [Hood *et al*(1); Menguy *et al*(2); Semb *et al*(3)]. A similar substance has been demonstrated in human saliva. The mechanism of transport of this gastric inhibitory substance is not clear—it is possible that it may be transported in lymph. Certain humoral substances, notably thyroid hormone [Daniel *et al*(4)], renin and an angiotensin-like substance [Lever and Peart(5)] have been demonstrated in the lymph.

The present study was undertaken to ascertain whether G.I.S. or a similar substance was present in autogenous thoracic duct lymph.

Methods and materials. Collection of lymph. Five Heidenhain pouch dogs weighing between 12 and 22 kg were utilized. The thoracic duct was cannulated with polyethylene cannulae both cranially and caudally,

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utilizing a lower right thoracotomy. The cannulae were connected extracutaneously, affording a duct-to-duct fistula, thus preventing dehydration, hypoproteinemia and lymphocyte depletion.

Lymph collections were carried out 2 to 3 days later when the animals had recovered from the effects of anesthesia. After an 18-24-hour period of fasting, lymph was collected for 2 hours into a sterile receptacle containing 0.5 ml heparin.

Separation of lymph. Five volumes of chloroform-methanol (2:1 vol/vol) were added to one volume of lymph. After stirring for one hour, the solution was centrifuged for 20 minutes, 3 layers emerging: (i) Aqueous methanol layer, clear and light yellow in color (Fraction I); (ii) Intermediate white layer (Fraction II); and (iii) A clear lipid-chloroform layer at the bottom (Fraction III). The top and bottom layers were removed by suction and evaporated to dryness. Each residue was redissolved in distilled water, dialyzed for 48 hours, and lyophilized.

After washing with ether, the intermediate layer was dried. It was redissolved in 0.025 N NaOH at pH 11.0 and stirred for 2 hours. The pH of the solution was adjusted to and maintained at pH 7.0 with addition of HCl. After centrifugation, the pH was lowered to pH 5.0, when the solution was further centrifuged. (It was noticed that maximum precipitation occurred at pH 5.0, the precipitate going into solution at pH 4.0-4.5.) The resulting precipitate was washed with ether and dried. The soluble fraction was adjusted to pH 7.0 with NaOH, then dialyzed and lyophilized.

Immeriately prior to use, the materials used for bioassay in each fraction were dissolved in normal saline and brought to pH 7.0. Any remaining insoluble material was filtered before injection into the animal.

Secretion studies. The assay of gastric secretory factors in the lymph was carried out by the method of Code *et al*(6). Following 18 control experiments with histamine alone, one-fourth or less of each lymph fraction was injected intravenously over a period of 30 minutes while the dogs were secreting

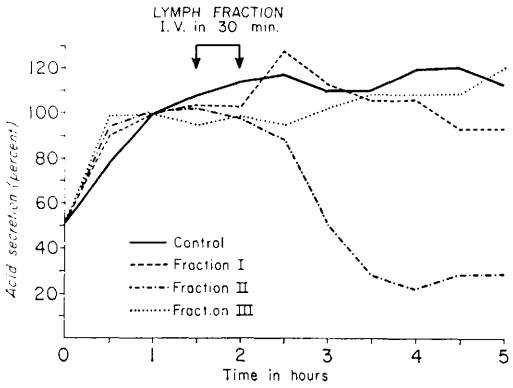

FIG. 1. Effects of all 3 fractions of lymph and histamine alone (control) on Heidenhain pouch acid output. Basal secretion (30-min collection) is regarded as 100%. The intermediate fraction (Fraction II) produced an 80% reduction in acid secretion.

from their Heidenhain pouches at a steady rate under the influence of a continuous intravenous infusion of histamine (50 to 200 μg histamine base per hour). Each fraction was studied 10 times. Gastric juice was collected from the Heidenhain pouches at 30-minute intervals; content of hydrochloric acid was determined against 0.01 N NaOH to pH 7.0.

Rectal temperatures were determined before and at intervals during all assays. Systemic reactions were very rare—on only 2 occasions did nausea or vomiting occur after injection of a lymph fraction.

Results. The results are summarized in Fig. 1. The 30-minute acid output while the Heidenhain pouch was secreting at a steady rate is regarded as 100%, and any stimulation or inhibition is expressed as a percentage of this. It will be noted that the fraction from the aqueous methanol layer (Fraction I) did not affect secretion. The fraction from the lipid-chloroform layer (Fraction III) on 3 occasions resulted in mild stimulation, but a similar effect was also seen with histamine alone. Profound inhibition was produced by the soluble fraction of the intermediate layer (Fraction II)—acid gastric secretion was reduced by 80%. Inhibition usually commenced after one hour, was maximal after 2 hours, and lasted for at least 4 hours (Table I).

Discussion. The results of this study demonstrate that a substance which inhibits exo-

TABLE I. Effect of Intravenously Administered Fractions of Fasting Lymph on Histamine-Stimulated Heidenhain Pouch Output.

	No. exp	Basal hr Avg mEq HCl	2nd hr post-injection Avg mEq HCl	2nd hr post-injection "t" test	3rd hr post-injection Avg mEq HCl	3rd hr post-injection "t" test
Fraction I	10	1.24	1.29	N.S.*	1.13	N.S.
" II	10	1.51	.37	$p = .001$	.43	$p = .003$
" III	10	1.45	1.62	N.S.	1.71	N.S.
Control (histamine alone)	18	1.77	1.88	N.S.	2.04	N.S.

* N.S. = Not significant.

genous gastric secretagogues is present in the thoracic duct lymph of dogs. The pattern of inhibition closely simulated that of G.I.S. previously found in this laboratory [Semb *et al*(3)]. The occasional stimulatory effects of the lipid-chloroform layer were possibly due to the histamine alone or to the gastric secretagogue activity of lipid derivatives. MacIntosh and Krueger(7) demonstrated that intravenous administration of choline and lecithin increased gastric-acid secretion from a Heidenhain pouch.

Previous studies of factors affecting gastric secretion in lymph have yielded conflicting results for several reasons. Apart from the study by Kelly *et al*(8), homologous lymph has been used. We have found that high speed centrifugation alone, as used by Johnson and Code(9), results in inadequate separation of the lipid from the non-lipid fractions of the lymph. Varying results might be expected if such fractions or, indeed, whole lymph were infused, depending on the preponderance of stimulatory or inhibitory factors. Administration of a physiologically abnormal amount of lymph, *e.g.*, a 2-hour collection over a period of 10-20 minutes, might conceivably result in either stimulation or inhibition. In our study, autogenous lymph was used in each case, thus eliminating almost entirely adverse reactions to foreign materials, and the dose used was physiological, *i.e.*, one-fourth of each 2-hour specimen was administered over a period of 30 minutes.

While the presence of a gastric inhibitor has been demonstrated in fasting lymph, it is not clear whether this substance is present in the lymph after a meal, and, if so, whether in the same quantities. The factors which release or stimulate its production are not known. Although this inhibitor has an action similar to G.I.S., further purification and identification will be necessary to determine whether this is, indeed, the same substance.

Gastric secretion may be inhibited by secretin, pancreozymin or cholecystokinin. The inhibitor fraction was tested for and found to have no secretin, pancreozymin or cholecystokinin activity.

Summary. These studies demonstrate the presence, in fractionated thoracic duct lymph, of an inhibitor of exogenous secretagogues. This inhibitor does not appear to be secretin, pancreozymin, or cholecystokinin, and its pattern of action resembles G.I.S. Further studies need to be undertaken and, if the agent is G.I.S., it may be possible to identify this inhibitor in thoracic duct lymph using such methods as chromatography.

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