not inhibit phagocytosis in the few instances in which it was tested.

Discussion and conclusions. This report is intended to describe a new observation. Speculation about the ultimate significance of virtually complete inhibition of leukocytic phagocytosis in hypergammaglobulinemic multiple myeloma (M.M.) plasma *in vitro* is clearly unwarranted at present, but a few tentative conclusions are justified: (1) Depressed phagocytic activity is directly attributable to the γ globulin fraction in M.M. plasma and not to a cellular defect. (2) Plasma from M.M. patients with hyperproteinemia due to increased levels of α or β globulins, or from patients with hypergammaglobulinemia due to disease other than M.M. does not inhibit leukocyte phagocytic activity. (3) Inhibited phagocytosis appears not to be solely a function of total gamma globulin level. This is most clearly demonstrated by the relatively low levels of γ globulin which inhibit phagocytosis in plasma of M.M. patients without hypergammaglobulinemia or hyperproteinemia; most notably when γ globulin levels are lower than normal. (4) Since the inhibitory effect of hypergammaglobulinemic M.M. plasma may be decreased or abolished by diluting the abnormal plasma with normal plasma or isotonic saline, the effect is not due to a simple deficiency. Specifically, the inhibitory phenomenon appears not to be due to a deficiency of normal gamma globulin. (5) Studies of the phenomenon are continuing.

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Some Characteristics of Para-Mucus Secretion.* (26125)

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It has been reported from this laboratory that the topical application of iodoacetamide (IAA) or N-ethyl maleimide (NEM) solutions to the mucosa of canine gastric pouches will evoke a flow of mucinous secretion for many hours(1). This material had never been recognized before, and because its physiological significance is still unknown, it was designated "para-mucus"(2). Exploratory experiments on the pepsin content of para-mucus indicated that the zymogen is present in appreciable quantities, and that it is probably a significant component in chemical characterization of this mucinous secretion. This report presents a systematic study of the variations in pepsinogen content of para-mucus, using specimens collected fractionally every 30 minutes for 6 hours following topical application of IAA solution to the gastric mucosa in dogs with a Heidenhain pouch and antrectomy. Several other characteristics of this material are presented here.

Material and method. Four Heidenhain pouch dogs, provided with an antrectomy, were used in these experiments. The antral resection was performed to insure the virtually complete absence of HCl secretion in the fasting state, in accordance with previous experience in this laboratory(3). Each dog was subjected to the following 2 types of experiment: A) *Histamine stimulation*: Dogs were starved for at least 18 hours before experiment, and unstimulated (basal) secretion was collected for 2 hours, at 60-minute intervals. Following this, histamine was administered subcutaneously (0.1 mg of histamine

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TABLE I. Volume-Rate of Flow and pH of Basal and Histamine-Stimulated Gastric Secretions.

Dog No.	Basal secretion		Histamine-stimulated secretion			
			1st hr		2nd hr	
	Vol-rate* (ml/hr)	pH	Vol-rate (ml/hr)	pH	Vol-rate (ml/hr)	pH
326	1.3	7.2	6.8	1.0	1.8	1.5
334	1.9	7.2	2.1	6.6	1.5	7.0
	1.0	7.4	2.6	3.5	2.0	6.8
	1.0	7.2	4.0	3.5	2.5	6.8
	1.0	7.0	4.5	3.5	1.3	6.5
340	.8	7.2	7.2	1.0	3.6	1.9
	.8	7.0	13.5	1.0	8.5	1.0
	1.3	7.0	10.5	1.0	3.2	1.0
382	1.4	7.5	8.3	1.0	5.5	1.3
	1.3	7.0	3.8	1.2	4.4	1.4
	1.8	7.2	10.0	1.0	3.0	3.5
Mean	1.2 (.8-1.9)†		6.66 (2.1-13.5)		3.39 (1.3-8.5)	

* Mean volume-rate over a 2-hr interval.
† Range.

base/kg body weight) and the acid secretion was collected for two 60-minute intervals thereafter. Such experiments were done to be sure that the acid-secretory behavior of the dogs was normal. B) *Topical application of IAA*: IAA in water solution, at a concentration of 3mM (55.5 mg/100 ml) was applied topically for 30 minutes, and secretion was collected every 30 minutes for 6 hours (12 experiments). The technics were those described previously(1).

For each specimen, volume, pH (electrometric), and pepsinogen (hemoglobin substrate method of Anson and Mirsky, as modified by Bucher) were determined as usual. Activity of the enzyme was expressed as meq. tyrosine $\times$ 10^4-designated Hb-units. Total and organic solids were also determined in 4 of these experiments, using the method previously described(4).

Observations and comment. Basal secretory activity. Volume-rate of secretion during basal conditions ranged between 0.8 and 1.9 ml/hour, with an average of 1.2 ml/hour for 2 hours of collection (Table I). There were no striking differences among the several dogs as to volume secreted, size of pouch, or lapse of time after operation. pH of these basal specimens never fell below 7.0.

Response to histamine stimulation. In 3 of the dogs, histamine produced the usual pattern of acid secretion during the first 2 hours after injection (Table I). In the fourth animal (dog #334), the volume secreted during 2 hours after histamine was markedly lower than for the other 3 averaging only 2.1 times as great as during basal secretion, in contrast to a 5.2-fold difference for the others. The pH of this dog remained above 3.5 in 6 of the 8 hourly collections, suggesting some abnormality of acid secretory function in this animal. Even doubling the histamine dosage failed to evoke any marked output of free acidity.

Volume and pH response after IAA. During the first 3 half-hours after topical application of IAA, volume-rates of para-mucus secretion were extremely high as compared with such data for basal and even acid secretion after histamine stimulation. For this interval, the rates varied in the range 4.6-11.5 ml/half hour with a mean of 7.2 for all 12 experiments. Since the responses to IAA of dog #334 were consistent with those for the other 3 animals, they were included in this average. By plotting the average volume-rate of para-mucus secretion, in ml/half hour, as a function of time following IAA stimulation and drainage (Fig. 1), it appears that the data conform roughly to a straight line with negative slope. For the sixth hour, mean volume-rate is still nearly 4 times that observed during the basal period, suggesting a persistence of IAA activity for at least this length of

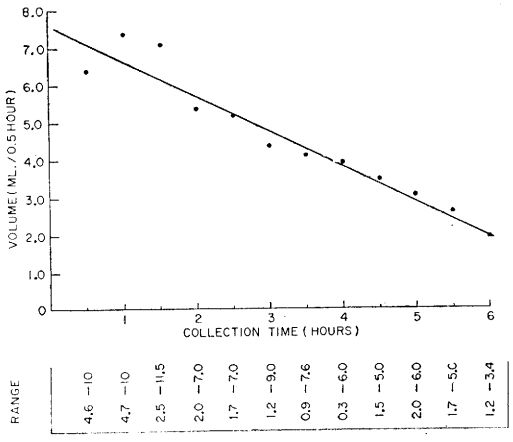

FIG. 1. Volume-rate of secretion of para-mucus as a function of time following topical application of iodoacetamide solution for 30 min. (mean of 12 exp. with 4 Heidenhain pouch dogs).

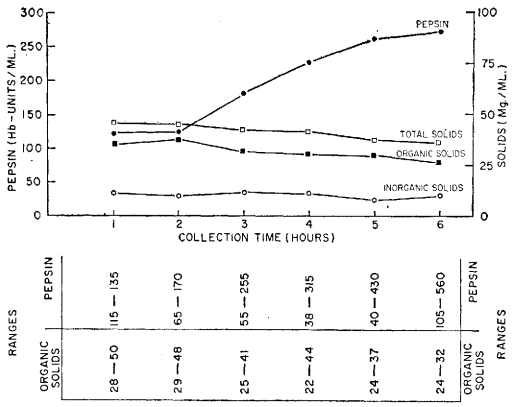

FIG. 2. Concentrations of several constituents of para-mucus as functions of time following topical application of iodoacetamide solution for 30 min. (mean of 12 exp. with 4 Heidenhain pouch dogs).

time, and probably one or more hours longer.

As previously reported(1), the pH values varied in the range 7.0-7.4 for all 30-minute specimens of the 12 experiments. These minor variations were not correlated with volume-rate of secretion nor with time of collection.

Because of the possibility that the secretory response to topically applied mustard oil (allyl iso-thiocyanate) bears some relation to that following stimulation with IAA, volume-rate curve for this mucinous secretion (5) was compared with that in Fig. 1. Whereas the latter approximates a rectilinear relation, the former was semi-logarithmic. The highest initial volume-rate of secretion following mustard oil administration was 17.4 ml/15 minutes; the mean for 15 experiments was 11.1 ml/15 minutes. These rates are equivalent to 34.8 and 22.2 ml/half hour respectively, the latter value being over 3 times as great as the mean rate (7.2) observed during the first 3 half-hour collection periods in the present experiments.

Organic and inorganic solids in para-mucus. In 4 of the experiments in which a single topical application of IAA was employed, the contents of total organic and inorganic solids were determined in each hourly sample. The resulting data, expressed as concentrations in terms of mg/ml secretion, are summarized in Fig. 2 as a function of collection time. The concentration of inorganic substances remained practically constant around a mean level of 9.6 mg/ml (unweighted for volume) throughout the 6 hours of experiment. Range for all 24 specimens was 5-14 mg/ml. Concentrations of organic and total solids, however, showed a consistent downward trend, starting with the second hour of collection; this pattern was followed by the 4 individual experiments as well as the curve for their average. For total solids the range of these 6 mean values reflects a mean change of 22%; for organic solids alone it is 26%. The individual values comprising these means varied from 22 to 50 mg/ml for organic solids, and from 34 to 57 mg/ml for total solids. Hourly outputs of all 3 of these factors, plotted as mg/hour against collection time (Fig. 3), reveal an even sharper descending line than do the corresponding concentration data of Fig. 2. Mathematically, this finding implies a definitive correlation with collection time, but also with volume-rate of secretion as is evident by comparison with the histogram for the latter. Whether the correlations for output of solids/hour with volume-rate/hour have physiological significance beyond that indicated by the corresponding concentration graphs, or whether they are entirely spurious, cannot now be established.

Concentration and rate of secretion of pepsinogen. A) Basal secretion: Pepsinogen concentration in the fasting secretion from 10 experiments ranged between 15 and 120

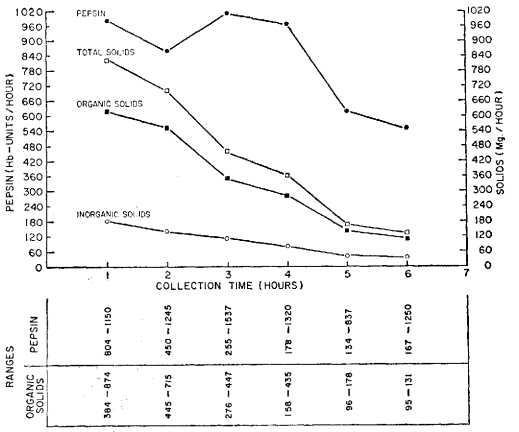

FIG. 3. Output-rates of secretion of several constituents of para-mucus as functions of time following topical application of iodoacetamide solution for 30 min. (mean of 12 exp. with 4 Heidenhain pouch dogs).

(mean 62) Hb-units/ml. Because of the low volumes of juice, however, rates of zymogen secretion (outputs) were also very low, varying in the range 8-94 (mean 40) Hb-units/half-hour. These data reflect the average behavior over 2 hours of observation.

B) After histamine stimulation: In the 3 dogs which gave acid secretion (pH in the neighborhood of 1), both concentrations and outputs of pepsinogen showed striking increases after injection of this stimulus. Thus, for individual hourly samples of all 10 experiments, concentrations were 3 or 5 times as great as those in the related basal secretion, though occasional specimens of histamine secretion had less than control values. The highest such concentration was 225 Hb-units/ml; the lowest was 25. Because of the great differences in volume-rate of flow after histamine, the data for output/hour showed even more striking rises over pre-injection levels. Thus, 10 to 35-fold increases were obtained in a number of the specimens, and in no case was post-injection level less than that for the basal secretion. The over-all range of post-histamine output values in the 3 good secretors was 184-2000 Hb-units/hour. In the fourth dog—the poor secretor for which the pH never fell below 3.5—individual pepsin concentrations and outputs following histamine were generally lower than in the other 3 animals, but the various ratios were in no way different.

C) After IAA application: Concentration of pepsin (Fig. 2) rose progressively for the 6 hours following drainage of the IAA solution—except from the first to the second hour—while volume-rate of secretion decreased steadily. During the fifth and sixth hours, when the pepsin curve started to flatten out, the enzyme concentrations averaged more than double those in the first 2 hours. The range of individual values in the 24 specimens making up this graph was 38-560 Hb-units/ml. Output of enzyme per hour (Fig. 3) decreased but slightly (if at all) during the first 4 hours, but thereafter began to drop significantly. The over-all range of variation among individual hourly specimens was 134-1755 Hb-units/hour. The curve for this variable appears not to be closely related to

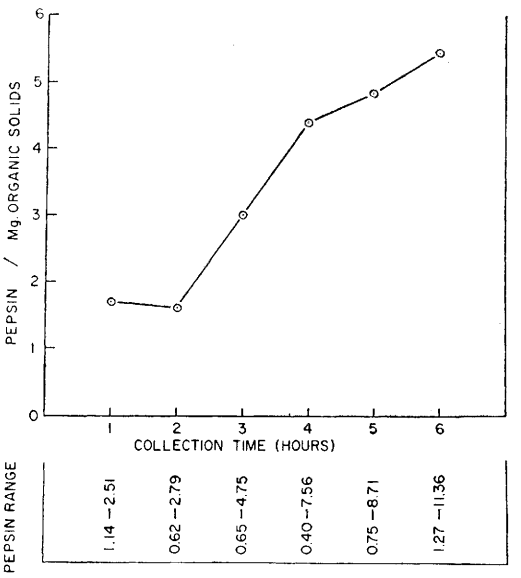

FIG. 4. Pepsin activity/mg organic solids as a function of time following topical application of iodoacetamide solution for 30 min. (mean of 4 exp. with 4 Heidenhain pouch dogs).

the corresponding one for output of total solids in the same figure, which suggests that pepsinogen probably comprises only a minor fraction of total content of organic substances in the IAA para-mucus. However, output ratios for pepsinogen to organic solids (corresponding to quantity of potential peptic activity per mg organic substances) increases steadily with time. The time curve for this ratio (Fig. 4), derived as averages from the individual ratios, conforms generally to the pattern of time-curves for enzyme concentration per ml of secretion in Fig. 2. This similarity results from the nearly constant character of the concentration of organic solids in Fig. 2. For the individual samples, ratios varied in the range 0.40 to 11.36 Hb-units/mg organic substances. Even in the low-acid secreting dog (#334), pepsinogen output and concentration after IAA stimulation were similar to those in the other 3 dogs. The individual graphs representing pepsinogen output as a function of time were similar for these 3 animals; only the low secretor showed an elevation at the second, third and fourth hours, apparently as a delayed response to the stimulus.

Comparison of all these data for the 4

dogs with corresponding values for basal and histamine-stimulated specimens revealed that outputs of pepsin for the mucinous secretion collected during the first 2 hours after IAA application were considerably higher (at least 2-fold) than for these other types of secretion. This observation may be significant in regard to the problem of whether histamine acts as a stimulus to pepsin as well as to acid secretion, or whether the enzyme is merely "washed out" by the parietal fluid. A study of enzyme response to histamine injected simultaneously with topical application of IAA may supply important evidence toward solving this problem.

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Ultrastructural Abnormalities In Whipple's Disease.* (26126)

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Whipple's Disease has been recognized with increasing frequency as one of the clinical entities associated with intestinal malabsorption. The underlying etiology has remained obscure but the histopathology of this disorder has been defined more accurately as a result of a variety of technical advances. Whipple(1) noted a prominence of fatty deposits in extracellular spaces as well as within tissue mononuclear cells. He also found many macrophages with foamy cytoplasm which did not stain for fat. Black-Schaffer subsequently demonstrated that these latter cells stain strongly with the periodic acid-Schiff (PAS) stain(2). His observations have been amply confirmed, and the occurrence of these PAS positive cells in the connective tissue throughout the body has been appreciated(3,4,5).

The introduction of peroral biopsy instruments(6,7) has made possible the histologic examination of normal and diseased small intestinal mucosa unaltered by post-mortem autolysis. This technic also permits tissue to be obtained rapidly enough for fixation and preservation of the fine structure for electron microscopic studies. This is a preliminary communication of light and electron microscopic findings in an intestinal biopsy from a patient with previously proven Whipple's Disease.

Methods. A fasting jejunal biopsy was obtained by means of a Crosby capsule from a 65-year-old male with known Whipple's Disease.† Portions of the specimen were fixed within one minute after biopsy in buffered osmium tetroxide(8) and in Dalton's solution (9), then dehydrated and embedded according to standard procedures(10).

Portions of the biopsy were also fixed in formalin for light microscopy. Sections of the osmium fixed as well as formalin fixed material were stained with the periodic acid-Schiff reagent. Thin sections were examined in an RCA-EMU 2B electron microscope at original magnifications of 1200 to 10,000 ×.

Results. By light microscopy the lamina propria of the biopsy specimen was found to contain a dense population of macrophages filled with PAS positive granules (Fig. 1). The granules appeared to be smaller in the cells adjacent to the lining of the epithelial

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† This patient was Case 46112, Case Records of Mass. Gen. Hosp., *New England J. Med.*, 1960, v262, 572.