Pepsin Secretion and Synthesis *in vitro*.* (20502)

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In vitro preparations of organs can be used to study secretion and synthesis of enzymes, and the purpose of the work reported here was to determine whether pepsin is synthesized by the isolated stomach. Edwards and Edwards (1) measured the secretion of pepsin by the toad gastric mucosa, but because they did not measure pepsin contained in the mucosa the synthesis of pepsin was not observed. Hokin (2) stated he was unable to obtain pepsin synthesis *in vitro*, but he gave no details.

Methods. Stomachs of mice of the C57 Black strain were used. They were prepared by the methods used for studying acid secretion *in vitro* which have been described in detail(3,4). The stomachs were filled to an internal pressure of 25 or 50 cm H_2O with isotonic, phosphate-buffered salt solution containing 20 mM glucose. They were placed in 4 ml of the same solution and incubated with shaking at 38°C under a pO_2 of 3200 mm Hg. After incubation the fluid within the stomach was removed, and its pH was measured. Acid secreted was estimated by multiplying the volume of the fluid by the number of micromoles of acid required to reduce the pH of a unit volume of the solution from its original to its final pH. The pepsin activity of the solution was measured after adjustment to pH 2 by the hemoglobin method of Anson and Mirsky(5). In the course of analysis all pepsinogen was converted to pepsin. The wet weight of the glandular portion of the stomach with the forestomach and the stumps of the duodenum and esophagus trimmed away was obtained. This tissue which consisted of both mucosa and muscularis was extracted by thorough grinding in 0.1 M acetate buffer at pH 4.5, and pepsin content of the extract was measured after adjustment to pH 2. Stomachs not incubated were also extracted. The analytical method was standardized using 2X recrystallized swine pepsin (Cudahy), and the results were expressed as mg pepsin per g wet weight of tissue.

Results. Stomachs were filled to an internal pressure of 50 cm H_2O and incubated for one hour in salt solutions containing various concentrations of carbaminoylcholine. Their rates of pepsin secretion are shown in Fig. 1. At zero drug concentration there is a basal rate of secretion. This basal rate is stimulated in part by distention of the stomach and in part by unknown factors. The maximal rate of secretion occurs at a drug concentration of 0.1 mg %, and at higher drug concentrations pepsin secretion falls toward the basal rate. The response of pepsin secretion to the parasympathomimetic drug closely parallels the response of acid secretion(3) which has a basal rate at zero drug concentration, a maximal rate

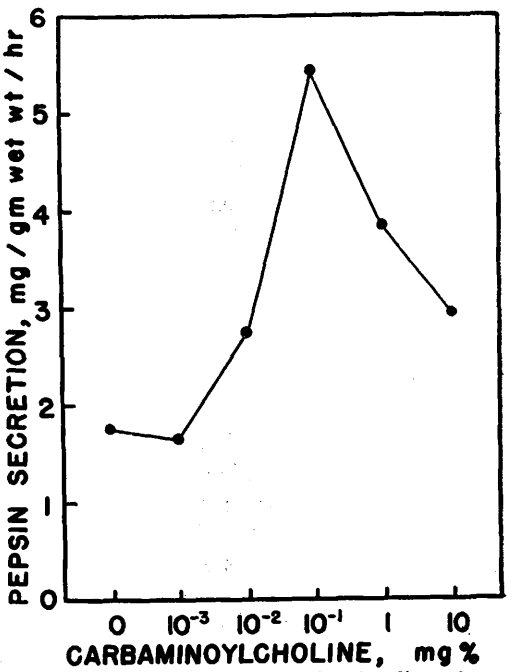

FIG. 1. Stimulation by carbaminoylcholine of pepsin secretion by mouse stomachs *in vitro*. Each point represents the mean of 5 or more observations.

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TABLE I. Pepsin Secreted by or Contained in Mouse Stomachs. (mg pepsin/g wet wt.)

Hr of incubation	Substrates	Total pepsin secreted, mean ± S.E.*	Pepsin in stomach, mean ± S.E.*
0	—	—	12.5 ± .4
1	Glucose	2.9 ± .1	9.7 ± .4
4	"	5.4 ± .3	5.4 ± .3
4	" + casein hydrolysate	—	5.6 ± .3
4	" + amino acid mixture	—	4.7 ± .2

* Stand. error of mean: $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$.

at 0.1 mg % and which falls off at higher concentrations. In 30 instances in which rates of both acid and pepsin secretion were measured on the same preparation the correlation coefficient between the variables was +0.84.

Atropine sulfate in concentrations of 0.01, 0.1 or 1 mg % has no effect upon the basal rate of pepsin secretion, but when carbaminoylcholine is also present these concentrations of atropine reduce the rate of pepsin secretion to the basal rate but not below it. Histamine in a concentration of 1 mg % increases the rate of pepsin secretion to about 50% above the basal rate.

In order to determine whether pepsin is synthesized *in vitro* the sum of the amount of pepsin secreted and contained in the stomach after incubation was compared with the amount present in unincubated stomachs. Results of these experiments are given in Table I. The pepsin content of stomachs not incubated is given in the first line. Stomachs filled to 25 cm H_2O internal pressure and incubated one hour in the presence of 0.1 mg % carbaminoylcholine and 20 mM glucose contained less pepsin at the end of incubation, but the sum of this pepsin plus that secreted equalled that in the control stomachs. To see whether the total pepsin would increase during a longer period of incubation stomachs were incubated under the same conditions for one hour, emptied, refilled and again incubated for an hour. The process of emptying and refilling was repeated for a total of 4 hours, and the total pepsin secreted and contained in the stomach was measured. The amount secreted in the 4 successive hours was 2.9, 1.2, 0.7 and 0.6 mg per g wet weight. The sum of that secreted and contained in the stomach was less than that present in the control stomachs. The differences can be accounted for by the losses occurring during the process of emptying and refilling. It is clear that no synthesis of pepsin from endogenous precursors occurred. The experiments were repeated with two different amino acid mixtures added to the incubation fluid. These were 0.2% of a tryptic digest of casein and a 0.15% mixture of the 19 amino acids occurring in pepsin(6) made up in the proportion in which they exist in the enzyme. Because it is not possible to use the Anson and Mirsky pepsin method on solutions containing tyrosine and because other pepsin methods are not sufficiently sensitive or accurate, the pepsin secreted in the presence of the amino acid mixtures could not be measured. In neither instance was the amount of pepsin found in the stomach at the end of 4 hours incubation greater than that found when only glucose was the substrate.

Discussion. Mouse stomachs contain an average of 12.5 mg pepsin per g wet weight. The tissue on which the weight was determined is approximately 56% mucosa and 44% muscularis(7). Therefore the pepsin content of the mucosa is about 23 mg per g wet weight. This is much higher than the figure of 6.4 mg per g wet weight for swine fundic mucosa derivable from the figures given by Herriott (8). The dry weight to wet weight ratio of the mouse stomach is 0.25, so that about 9% of the solids of the mucosa is pepsin or pepsinogen.

The conditions under which the stomachs were incubated are optimal for acid secretion (3), but they are not adequate for pepsin synthesis. Pepsin may not be synthesized because the chief cells are damaged by the *in*

vitro conditions or because the correct precursors were not supplied. The facts that the parietal cells function under these conditions and that the histological appearance of the stomach is good after incubation(9) make it doubtful that the chief cells are seriously injured. Hokin(2) in the same paper in which he notes he was unable to obtain pepsin synthesis shows that a mixture of amino acids or 0.2% casein hydrolysate supports enzyme synthesis by pancreatic slices *in vitro*. The stomach may require more complex substances than amino acids for pepsin synthesis, but there is no evidence indicating the nature of these requirements.

The fact that pepsin synthesis does not occur under the conditions described has an important bearing on the calculation of the efficiency of gastric acid secretion. The oxygen consumption and acid secretion by mouse stomachs have been measured under nearly the same conditions(10). The efficiency of the acid-secreting process in terms of moles of hydrogen ions secreted per mole of oxygen used for acid secretion has been calculated from the observed increases of both variables upon stimulation by 0.1% carbaminoylcholine. The efficiency has been found to be about 2 moles of acid per mole of oxygen. If some of the additional oxygen used after stimulation of the stomach were used for pepsin synthesis rather than for acid secretion the calculated efficiency would be lower than the true efficiency. Because no pepsin is formed, no correction need be applied for the process. However, pepsin secretion is stimulated by the drug, and if any appreciable amount of oxygen is used for pepsin secretion the calculated cost of acid secretion should be corrected for this.

Summary. Pepsin secretion and synthesis by mouse stomachs was studied *in vitro*. There is a basal rate of pepsin secretion, and carbaminoylcholine stimulates additional secretion. At the optimal drug concentration of 0.1 mg % the rate of secretion is 300% of the basal rate. Atropine inhibits the additional secretion caused by carbaminoylcholine but not the basal secretion. Histamine slightly stimulates pepsin secretion. The stomachs were supplied with amino acid mixtures, but no pepsin synthesis could be found. The implications of failure to find synthesis are discussed.

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