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Experimental Hyperplasia of the Stomach Mucosa.

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The mucosa of the human stomach presents striking individual differences in area and in thickness.[1] These are often unrelated to any distinct qualitative differences in mucosal structure. It has been noted in further studies in this laboratory that the thickness of the mucosa of the specific secretory portion of the human stomach is particularly subject to marked individual difference, and that there is a roughly parallel diversity in the number of parietal, or acid secreting, cells. This variation has been seen to occur in stomachs which would otherwise be classified as normal, and it has suggested the possibility that stomachs with large numbers of secreting cells may be instances of hyperplasia associated with increased functional activity.

Because of numerous demonstrations of increases in number and size of structural elements of many different organs in association with increased function, it should be expected that gastric mucosa might undergo hyperplasia, if its secretory activity is increased. The following experiment was designed to induce this state in guinea pigs by prolonged stimulation with injected histamine according to the method of Code and Varco.[2]

Methods. Fourteen young guinea pigs of both sexes, ranging in weight from 310 to 830 g, were injected intramuscularly with 3 to 6 mg of histamine phosphate* in beeswax-mineral oil mixture 3 times weekly for from 2 to 4 weeks. The animals were sacrificed by bleeding while under ether anesthesia. The stomachs were removed immediately, opened along the greater curvature, stretched on cardboard to remove all mucosal folds, and then were fixed in 4% formaldehyde solution. A map of each fixed specimen was prepared by tracing the outline upon paper, and the location of 3 excised strips of gastric wall was plotted upon it. These strips were representative samples of the dorsal and ventral walls and lesser curvature. Longitudinal histological sections 8 microns thick were stained with Giemsa stain, and the amount of linear shrinkage of the strips during the preparation was determined. The junction of the acid secreting portion of the mucosa with that of the pyloric zone was identified in the sections, and after an appropriate correction, was plotted upon the map. A line joining the 3 points thus obtained, determined the approximate position of the junction between the 2 mucosal zones. The area of the acid secreting zone was measured in 3 parts with a planimeter: the ventral wall, the dorsal wall, and the lesser curvature. The latter was defined arbitrarily as the zone within lines joining the

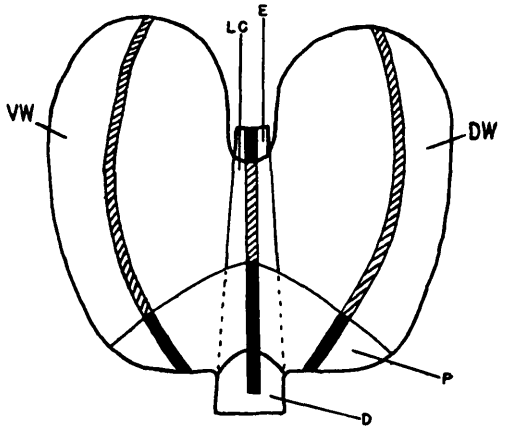

FIG. 1.

Diagram of an opened, stretched guinea pig stomach showing the 3 excised strips and the mucosal zones. The striped portions of the strips show the location of parietal cells. D = duodenum, E = esophagus, P = pyloric zone, VW = ventral wall, DW = dorsal wall, LC = lesser curvature.

1 Cox, A. J., *Calif. and Western Med.*, in press.

2 Code, C. F., and Varco, R. L., *Am. J. Physiol.*, 1942, **137**, 225.

* The histamine phosphate (Ergamine phosphate) was supplied in part through the courtesy of Burroughs Wellcome & Co.

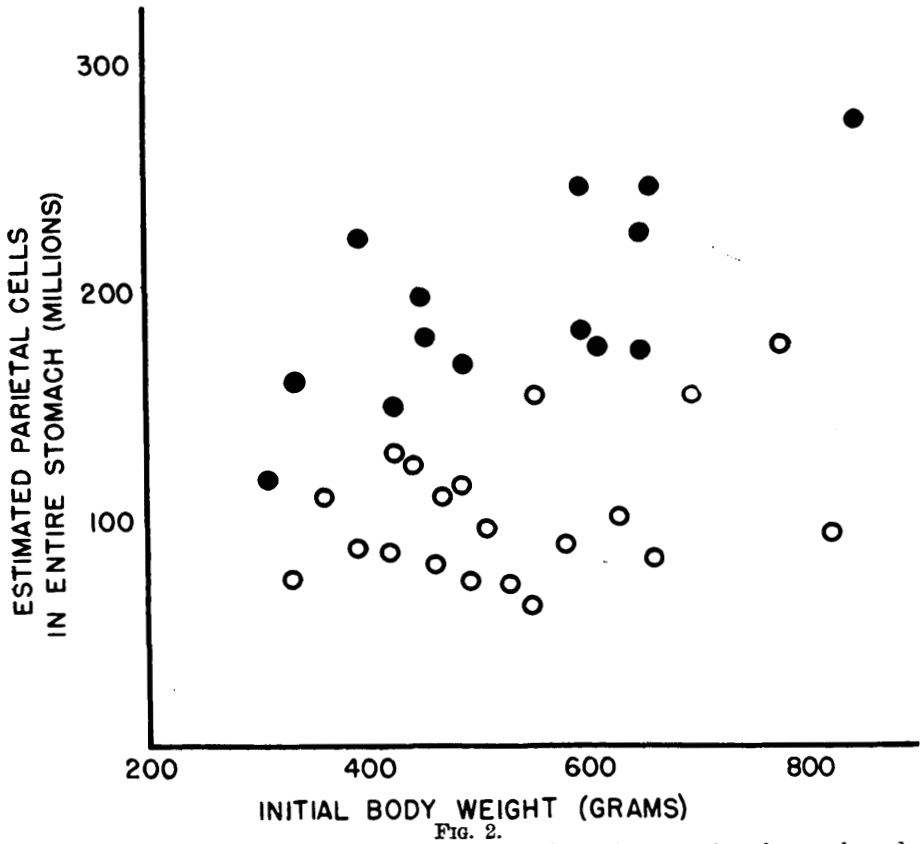

FIG. 2.

Estimated total number of parietal cells in guinea pig stomachs after prolonged administration of histamine. ● = treated animals; ○ = control animals.

edges of the opened duodenum with those of the opened esophagus (Fig. 1).

In each stomach, from the histological sections which were cut perpendicular to the mucosal surface, 36 counts were made of the number of parietal cells in representative bands 0.23 mm broad extending across the sections from the surface to the base of the mucosa. Only cells in which nuclei were visible were counted; this eliminated the necessity of identifying cells which were barely included in the section, and permitted the estimation of an "effective thickness" of the section by adding the average measured diameter of the parietal cell nuclei to the actual thickness of the section. This average nuclear diameter (5 microns) was the same in treated and control animals. From the representative counts and the measured areas, an estimated total count was calculated for each of the 3 regions mentioned above, after correcting for shrinkage in length of the specimen during preparation. By addition, an estimated total number of parietal cells for each entire stomach was obtained. No correction was made for shrinkage in width of the prepared strips of stomach wall, but this could not be determined accurately, and since shrinkage in length was essentially the same in the treated and untreated animals, it was assumed that shrinkage in width would also be similar in the two groups.

Results. The estimated numbers of parietal cells in the different stomachs are shown in Fig. 2. The controls were 20 animals of similar weight and sex, and in some instances were littermates of the treated animals. It can be seen that in the stomachs of the treated group, where it can be assumed that there had been prolonged increased secretion of acid,[2,3]

[3] Code, C. F., and Varco, R. L., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 475.

there were distinctly more parietal cells. No difference could be attributed to the sex of the animals, and the size of the injected quantities of histamine was not clearly related to the degree of response. However, the animals which were subjected to the longer series of injections showed somewhat greater increases in parietal cells than did those which were injected for only 2 weeks. Five additional similarly treated animals died with perforated pyloric or duodenal ulcers before completion of the injections. No animals with ulcers were used for estimation of parietal cell increases.

Estimation of the number of other cell types in the mucosa is more difficult than reckoning the parietal cells, which are usually clearly outlined and easily identified. Therefore, counts of other cell types have not been made.

Nothing was seen to suggest that the increase in number of parietal cells was due to any process other than true hyperplasia. No partially granulated parietal cells were recognized. It is probable that the increase in cells represents a response to stimulation of function.

Summary and Conclusion. An increase in number of parietal cells occurs in the mucosa of the guinea pig stomach after protracted stimulation with histamine over a period of 2 to 4 weeks. This is presumably a hyperplasia and may indicate a mechanism to explain differences in the number of secreting cells in different human stomachs.

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Effect of Digitalis on Cholinesterase.

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The mechanism through which digitalis brings about a slowing in cardiac rate has been the subject of a number of views. Abdon *et al.*[1] have suggested that the digitalis bradycardia might be explained as the result of a sensitization of the myocardium to acetylcholine, possibly through the inhibition of cholinesterase. These workers repeated certain experiments of Matthes[2] using a colorimetric method for the determination of acetylcholine, and were in agreement with him in finding no inhibition of the enzymatic breakdown of acetylcholine by *g*-strophanthin in concentrations up to 1:5000.

No data other than these summary statements of a negative result are given in these reports, and other references to the effect of digitalis on cholinesterase have not been found. Further evidence on this point has been obtained with a specific cholinesterase prepared from the electric organ of the eel as well as with non-specific human serum esterase.

The following digitalis glycosides were studied: digitoxin, ouabain, strophanthin, and lanatoside C. The effect of each of these substances was tested on both serum esterase and eel cholinesterase, using a concentration range approximating that which might obtain in the blood following a therapeutic dose, as well as higher concentrations. For the determination of cholinesterase activity the Warburg manometric technic was employed. Dilution of the glycosides was made in saline, and 0.5 cc of this was added to 3 cc of a 0.02 M solution of acetylcholine in the vessel. The acetylcholine solution was made by dissolving 225.9 mg of acetylcholine bromide in 50 cc of 0.03 M sodium bicarbonate. In the case of controls, saline alone in an equivalent amount was added to the flask. The enzyme dilution (0.5 cc) was placed in the side bulb and later tipped into the reaction mixture. The final dilution of human serum after tipping was 1:40, and of the eel esterase 1:3200.

1 Abdon, N. O., and Nielsen, N. A., *Skand. Arch. Physiol.*, 1938, **78**, 13.

2 Matthes, K., *J. Physiol.*, 1930, **70**, 338.