DISTRIBUTION OF ESTERASE IN GASTRIC MUCOSA*

By W. L. DOYLE

(From the Department of Anatomy, University of Chicago, Chicago)

(Received for publication, May 19, 1954)

Prior studies on the quantitative distribution of esterase and choline esterase in the gastric mucosa of the pig and rabbit have indicated that maximal activity occurred near the surface of the mucosa but there has been no detailed histological analysis of the samples. Thus in the pig fundus, Glick (1) reported esterase activity in interstitial tissue, neck mucous cells, parietal cells, zymogen cells, and maximal activity in the epithelial cells (foveolae). In the rabbit, Glick (2) found that choline esterase activity was highest in the "epithelial cell region" with no consistent profile of distribution at deeper layers.

The present study relates the esterase activity more precisely to the histological composition of selected levels of the rabbit fundic mucosa. It also gives quantitative values for the hydrolysis of phenyl benzoate used to estimate esterase activity.

A piece of fundus was excised under nembutal anesthesia and promptly frozen in isopentane at approximately −150°C. This material was vacuum-dried while frozen at −28°C. and embedded in paraffin with negligible loss of esterase activity. Sections of tissue 10 μ thick were cut parallel to the surface of the mucosa from blocks 2 × 2 mm. square and were used alternately for histological study and quantitative estimation of esterase activity. For histology the sections were secondarily fixed with Zenker-formol before removing the paraffin and subsequently stained with pyronin and methyl green. The volumes of the various cell types present were estimated by the method of Chalkley (3). Esterase activity was estimated by the phenylbenzoate method of Gomori (4) scaled down 100 times. This measures serum-cholinesterase as well as aliesterase as shown by Rider, Moeller, and DuBois (5).

The gastric mucosa in the rabbit has a surface epithelium of regular columnar mucus-containing cells. This epithelium is invaginated to form the gastric pits or foveolae at the bottom of which the gastric glands arise as one or two simple tubules in which are found four cell types, the parietal cells, the zymogen cells, the neck mucous cells, and scattered argentaffine cells. Where the necks of the glands open into the bottom of the foveolae the mucous neck cells are connected with the surface epithelium by a series of gradual transitional forms. The mucosa of the rabbit fundus is difficult to fix by freezing in

* This work was aided by a grant from the Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

a perfectly flat plane but areas 1 or 2 mm. square can be found which are adequately flat. In such pieces the thickness of the mucosa varies with the location and in the specimens analyzed in this study the mucosa was 0.72 mm. thick. The foveolae intruded approximately 0.16 mm. from the surface. In the fundic glands the next 0.16 mm. was predominantly composed of parietal cells and the deepest 0.40 mm. was primarily composed of zymogen cells. Between the glands there is the connective tissue of the lamina propria mucosae which constitutes 25 to 45 per cent of the volume of a section depending on the distance from the surface. The mucosa can therefore be roughly divided

TABLE I

Esterase Activity in Rabbit Fundus

Level from surface	Esterase activity, μM phenol per c.mm.	Volume composition				Corrected activity	Activity of neck mucous and foveolar cells
		Neck mucous and foveolar cells	Parietal cells	Zymogen cells	Connective tissue		
1	2	3	4	5	6	7	8
mm.		*per cent*	*per cent*	*per cent*	*per cent*		*c.mm.*
0.05	0.79	56	0	0	43	0.77	1.4
0.07	0.83	—	—	—	—	—	—
0.10	0.84	—	—	—	—	—	—
0.12	(0.91)	51.4	5.6	0	43	0.89	1.7
0.13	0.94	—	—	—	—	—	—
0.20	0.41	—	—	—	—	—	—
0.21	(0.33)	20.5	40.5	0	39	0.31	1.5
0.23	0.18	—	—	—	—	—	—
0.30	0.08	5.5	50.5	8.0	36	0.06	1.1
0.37	0.04	1.5 ± 0.5	44.5	24	30	0.02	1.3
0.52	0.02	0	13.8	59.2	27	0	
0.73	0.02	0	10.5	42.4	(Muscle)	0	

Values in parentheses are calculated from the adjacent levels. Several values from level 0.38 to 0.72 are omitted since the activity (column 2) was invariably 0.02 μM of phenol per c.mm.

into three layers according to the predominating epithelium; *viz.*, foveolae, parietal, and zymogen layers. These are, however, very crude designations and in cutting sections parallel to the surface of the mucosa it is not possible to obtain samples consisting of single types of epithelial cells. In the present series the purest sample was obtained in the parietal layer in which 80 per cent of the *epithelial* cells were parietal cells. In the necks of the gastric glands the parietal cells in sections analyzed varied from 10 to 50 per cent of the volume and in the zymogen layer most sections showed about 25 per cent of the volume of epithelial cells to be parietal cells.

Table I gives for a representative block of tissue a comparison of the morphological analysis with the observed esterase activity. The esterase values represent extracts of 10 micron sections (0.038 c. mm. volume) of frozen-dried

fundus. In each section there were 1330 ± 45 tubules in cross-section. In the specimens analyzed there was an abrupt change in activity at the upper levels of the parietal cell layer (levels 0.20 and 0.23). At level 0.13 approximately 10 per cent of the cells in the necks of the glands are parietal cells. Level 0.21 has 30 per cent parietal cells per tubule. From level 0.30 to 0.37 the tubules containing zymogen cells have approximately 25 per cent parietal cells. The esterase activity (hydrolysis of phenyl benzoate) was maximal at a distance of 0.13 mm. from the surface of the mucosa. This level cuts the bases of some foveolae and the necks of some fundic glands and contains the maximum number of foveolar and neck mucous cells. It also contains 43 per cent by volume of connective tissue. The observed esterase activity at this level was 0.9 μM of phenol liberated in 30 minutes at 30°C. per c. mm. of tissue. (By calculation this would be 1.7 μM per c. mm. of foveolar and neck mucous cells.) At a level 0.30 mm. from the surface there were maximal numbers of parietal cells (50.5 per cent by volume) and 5.5 per cent of neck mucous cells. This level had only one-tenth the esterase activity of the 0.12 mm. level. At 0.52 mm. where there were maximal numbers of zymogen cells (59 per cent by volume) there was approximately only one-fiftieth the esterase activity of the layer richest in foveolar and neck mucous cells.

From the table it is evident that a consistent low level of activity (0.02 μM per c. mm.) occurs when foveolar and neck mucous cells are absent. This activity may be assigned either to the epithelial cells or the connective tissue but, since sections at 0.52 mm. and 0.73 mm. have widely differing cell compositions with equal esterase values, if the activity is in the epithelial cells one must assume that parietal and zymogen cells contain equal and low concentrations. At any given level of the mucosa in which they are present it would appear that about 97 per cent of the esterase activity is attributable to the foveolar and neck mucous cells which are present. In the table, column 7 gives the activity corrected by assuming the value of 0.02 for the connective tissue. Column 8 gives this activity calculated per cubic millimeter of neck mucous and foveolar cells. The value found is not constant and its variation with position in the tissue (except for level 0.37) is believed to be valid. This variation would indicate that the foveolar cells and neck mucous cells nearest the base of the foveolae have the highest concentration of esterase. The lowest value for these cells (at level 0.30) is about two-thirds of the maximal value. The values for the volume of the lumen of the gland could not be estimated with any accuracy. Its actual volume would be negligible except possibly at 0.05 mm.

It may also be noted that esterase activity was completely extracted from sections in 20 minutes by M/30 phosphate buffer and was stable for several hours. Aliquots of samples at levels 0.05, 0.13, 0.23, 0.30, 0.37, and 0.52 mm. from the surface were tested for esterase activity using phenyl butyrate[1]

[1] Supplied by Dr. G. Gomori.

substrate in place of phenyl benzoate and in general there was three times as much phenol liberated. Experiments with eserine gave 50 per cent inhibition at a concentration of 5×10^{-5} M.

DISCUSSION

Although choline esterase of serum will split phenyl benzoate (5) and sections of gastric mucosa split acetylcholine (2), the present experiments do not indicate which esterase is being estimated but it is probably almost entirely aliesterase.

The connective tissue of the stomach mucosa contains the usual elements of the lamina propria mucosae including a high percentage of extracellular fluid. The serum esterase is a readily diffusible substance and is to be found in lymph and extracellular fluid in about the concentration necessary to account for the esterase activity of sections containing no foveolar or neck mucous cells. It seems likely therefore that the parietal and zymogen cells contain little or no esterase and that their esterase content does not exceed the extracellular fluid level. With this correction all the esterase of the stomach mucosa appears to reside in the foveolar and neck mucous cells of the gastric pits and glands. Positive staining reactions for esterase in rabbit parietal cells have been found by Nachlas and Seligman (6) using β-naphthyl acetate and in the canaliculi by Chessick (7) using the acetate of 2-hydroxy-3-napthoic acid (naphthol AS acetate) substrate. In view of the rapid extraction of esterase found by Doyle and Liebelt (8) these staining reactions presumably depend upon 10 per cent or less of enzyme residual in the tissue. Unless these authors were dealing with some very specific enzymes which do not readily split phenyl benzoate it must be concluded that the staining reactions in parietal cells or canaliculi are insignificant in a quantitative analysis of the tissue.

SUMMARY

Sections of frozen-dried stomach mucosa of the rabbit cut at various levels from the surface were analyzed for esterase activity in relation to the proportions of cell types present. Aside from the fraction attributable to extracellular esterase all the esterase activity appears to be in proportion to the numbers of foveolar and neck mucous cells present in the sample.

REFERENCES

1. Glick, D., *Compt.-rend. trav. Lab. Carlsberg*, 1935, **20**, série chimique, 57.
2. Glick, D., *J. Gen. Physiol.*, 1938, **21**, 297.
3. Chalkley, H. W., *J. Nat. Cancer Inst.*, 1943, **4**, 47.
4. Gomori, G., *J. Lab. and Clin. Med.*, 1949, **34**, 275.
5. Rider, J. A., Moeller, H. C., and DuBois, K. P., *Proc. Soc. Exp. Biol. and Med.*, 1951, **76**, 427.
6. Nachlas, M., and Seligman, A., *Anat. Rec.*, 1949, **105**, 677.
7. Chessick, R. D., *J. Histochem. and Cytochem.*, 1953, **1**, 471.
8. Doyle, W. L., and Liebelt, R., *Anat. Rec.*, 1954, **118**, 384.