STUDIES ON ENZYMATIC HISTOCHEMISTRY

XXVI. THE HISTOLOGICAL DISTRIBUTION OF CHOLINE ESTERASE IN THE GASTRIC MUCOSA NORMALLY AND AFTER ADMINISTRATION OF CERTAIN DRUGS*

BY DAVID GLICK **

(From the Carlsberg Laboratory, Copenhagen)

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In previous papers of this series Linderstrøm-Lang, Holter, and coworkers have investigated the histological distribution of pepsin (1, 2), acid (2, 3), peptidase (2, 4), and esterase (2, 5) in the gastric mucosa of pigs, and the urease (6) in dog’s stomach. The present investigation is a continuation of this work with regard to choline esterase. In view of the well known theory of chemical mediation of nerve impulses, choline esterase should be of particular interest in those organs, such as the stomach, which are influenced by the parasympathetic nervous system.

Dale and Feldberg (7) have demonstrated the liberation in the stomach of a substance indistinguishable from acetyl choline when the thoracic vagi were stimulated. These investigators pointed out the uncertainty regarding the point at which the acetyl choline is set free in the stomach. The possibility that it might be produced in the mucous membrane rather than in the muscle coats has been discounted by them since they observed large increases in output only when vagus stimulation threw the muscular wall into vigorous contraction. One would expect the region in which the acetyl choline is chiefly liberated to be the site of the greatest concentration of the enzyme hydrolyzing this substance, since choline esterase is an important factor in the physiological destruction of acetyl choline, a process believed to account for the short-lived effect of a nerve

* This paper also belongs to the series of investigations by the author and coworkers as “Studies in histochemistry. XIV.”

** Fellow of The Rockefeller Foundation.
Furthermore nervous tissue, which liberates acetyl choline upon stimulation, is the richest source of choline esterase in the animal body. In the present instance it has been found that by far the greatest concentration of the enzyme occurs in the epithelial cell layer of the gastric mucosa, and relatively little in the muscle. This would tend to lend weight to the possibility that vagus stimulation causes the acetyl choline to be liberated chiefly in the epithelial cell portion of the mucosa. Direct evidence, however, awaits future developments.

It was the purpose of the present investigation to determine the quantitative distribution of choline esterase through the stomach wall of the pig in normal fasting and fed animals, and in those which had been given a previous injection of acetyl choline, eserine, or atropine. A correlation was drawn between enzyme activity and the cellular composition of the tissue at any point.

**EXPERIMENTAL**

**Procedure for the Study of the Enzyme Distribution in Stomach**

The method of sampling the tissue, obtaining serial microtome sections of the frozen material, and the extraction of the enzyme were analogous to the procedures used for study of other enzymes (1 and 4-6). The tissue from the freshly killed animal was stored at -12° and the experiments were performed within 1 to 2 days. Under these conditions no demonstrable loss in activity occurred. Two consecutive circular sections, each 25 μ thick, and having a diameter of 2.6 mm. were placed in 9.2 cm. of 30 per cent glycerol in each reaction tube. After extraction at room temperature for 2 hours, 16 cm. of 1/10 veronal buffer containing 1/4 per cent acetyl choline chloride were added and the mixture was stirred by means of the magnetic “flea” in usual manner. The reaction was allowed to proceed at 30° for 1 1/4 hours, in the case of cardia and fundus, but for only 1 hour with pylorus tissue because of its greater activity. Enzymatic hydrolysis was brought to a standstill, and the titration carried out with Na/20 HCl to an end point at pH 6.2 according to the method already described (9).

Control experiments at 30° were conducted to determine the non-enzymatic splitting by placing the substrate-buffer mixture as a drop on the side of the reaction tube containing, but not in contact with, the glycerol solution with the tissue sections.

**Extractability of the Enzyme**

In order to be certain that the 2 hour extraction of the sections was sufficient, an experiment was performed in which alternate deter-
minations were made upon tissue extracted for 2 and 4 hours respectively (Fig. 1). From the manner in which the points lie on the curve it is apparent that complete extraction has occurred in 2 hours.

It is of interest to obtain some idea of the tenacity with which choline esterase is bound to the tissue. For this purpose an experiment was conducted in which estimations of activity were carried out alternately on sections treated with 30 per cent glycerol in the usual manner, and with 0.9 per cent NaCl solution for 2 hours. Fig. 2 shows the results of this experiment, and it may be seen that the physiological salt solution serves as well as the 30 per cent glycerol.

It should be borne in mind that the greatest dimension of the various stomach cells lies approximately between 10 and 20µ, and the 25µ sections of frozen tissue must contain a considerable percentage of cells that have been cut open. This may account in part for the ease of availability of the enzyme.

The possibility should not be overlooked that in the experiment described above the enzyme may not have been extracted at all, but the substrate may have diffused into the cells and around the portions of the cut cells. To demonstrate whether the enzyme is actually removed from the tissue, it would be necessary to separate the sections from the liquid medium, and then determine the activity of this liquid. This has been done in the following fashion:

Estimations of activity were conducted alternately upon sections treated with 30 per cent glycerol in the usual manner, and upon sections placed first in 0.9 per cent NaCl solution for 2 hours and subsequently in 30 per cent glycerol for the same length of time. In the latter case the sections were removed from the saline solution with a glass needle and transferred to separate tubes containing 9.2 c.mm. of 30 per cent glycerol. 6.5 c.mm. of the saline solution were used, and after removal of the tissue, 2.7 c.mm. of pure glycerol were added to make a final concentration of 30 per cent in a volume of 9.2 c.mm. The quantity of enzyme in this saline-glycerol solution was determined, and another determination was made on the glycerol solution with the transferred sections. The results of these experiments are represented in Fig. 3. The magnitude of the activities observed in the saline solutions is a little less than the actual activities since a film of the saline extract remains on the surface of the
sections when they are transferred to the glycerol solution. Most of the enzyme appears in the saline solution, and the sum of the activities observed in this and the subsequent glycerol solution equals, or exceeds to some degree, the activities obtained by the single glycerol treatment alone. It would appear from this that choline esterase in the source in question is easily diffusible under the experimental conditions chosen, and is extracted from the tissue even by physiological salt solution.

In this connection it is interesting to note that Dale and Dudley (10) observed a rapid disappearance of acetyl choline from spleen when the tissue was minced, though little of the substance was lost if the organ was allowed to remain intact until ground for analysis of acetyl choline. Apparently the mincing process brought the esterase and acetyl choline into contact with one another, from which it might appear that either the enzyme and substrate are normally separated within the cell, or that the disruption of the cell resulted in activation of the enzyme or formation of the substrate from some precursor or both.

**Histological Correlation**

In order to determine from which part of the mucosa the titrated serial sections were obtained, the tissue remaining after the sample had been removed for enzyme study was fixed, mounted, sectioned, and stained as described by Linderstrøm-Lang, Holter, and Søeborg Ohlsen (2). The cellular composition of the stained sections was correlated with the titration curves (2), and the latter were labeled accordingly (Figs. 1–13). The designations on the curves represent the positions where the respective cells occurred in greatest number. Cell counts were not performed in the present case since the epithelial cell region, which is obviously the one of greatest interest in relation to choline esterase, was rarely sufficiently intact in the stained sections to permit suitable counting.

**RESULTS**

**Experiments on Stomachs from Normal Fasting Animals**

The tissue used in these experiments was obtained from pigs which had been starved for 24 hours previous to killing. Figs. 2–4 demon-
strate the distribution of choline esterase in the cardia, fundus, and pylorus. In every case the highest enzyme concentration was found in the region where the epithelial cells predominate. In some instances a dip in the curve occurs beyond the epithelial portion followed by a rise in the chief cell region (Fig. 2). However, with other

![Graph](https://example.com/graph.png)

**Fig. 1.** On all the curves E refers to region of maximum concentration of epithelial cells; C refers to region of maximum concentration of chief cells; M refers to beginning of muscle layers; and P fundus refers to region of maximum concentration of parietal cells. Abscissae of all curves, millimeters from surface of mucosa. Ordinates of all curves, hydrolysis (c.mm. N/20 HCl after 1.5 hours in cardia and fundus, 1 hour in pylorus). Choline esterase distribution in fundus 88. Animal injected with 150 mg. acetyl choline chloride 1 hour before killing.

- ○ = sections extracted for 2 hours.
- × = controls.
- △ = sections extracted for 4 hours.
- ▲ = controls.

samples of tissue no dip was observed, but a steady decline followed to the region of the chief cells, and usually a further decrease in activity was demonstrable in the muscle tissue. The pylorus contained the enzyme in greater concentration than other parts of the stomach since practically as much activity was observed in 1 hour with this tissue as in 1.5 hours with the other tissues.
Experiments on Stomachs from Normal Fed Animals

The animals used for these experiments had been allowed to feed on their usual diet up until the time they were killed. The stomachs were found to be well filled with food. Figs. 5 and 6 demonstrate typical curves for this material. The curves are essentially the same as those obtained using stomachs from fasting animals.

Experiments on Stomachs of Animals Injected with Acetyl Choline

Acetyl choline chloride in freshly prepared aqueous solution was injected intramuscularly in doses of 100 or 150 mg. into the hindquarters of fasting animals 30 minutes and 1 hour respectively before the animals were killed. Reaction to the drug was apparent at the time the animals were slaughtered as indicated by excessive salivation and the presence of more than the usual amount of fluid in the otherwise empty stomach. The data obtained (Figs. 1 and 7-9) do not differ actually either qualitatively or quantitatively from those of the normal uninjected animals. The broad maximum shown in
Fig. 3. Choline esterase distribution in fundus 94. Normal fasting animal.
- \( \triangle \) = sections extracted with 30 per cent glycerol.
- \( \times \) = controls.
- \( \Delta \) = first extract with 0.9 per cent saline.
- \( + \) = subsequent extract with 30 per cent glycerol.
- \( \square \) = combined (\( \triangle \)) and (\( + \)).

Fig. 4. Choline esterase distribution in pylorus 82. Normal fasting animal.
In Figs. 4-13, \( \circ \) = total activity and \( \times \) = control.
Fig. 9 is the effect of the presence of an abnormally great number of epithelial cells throughout the tissue to a depth of 1.5 mm.

![Graph showing enzyme activity vs. distance from surface of mucosa.]

**Fig. 5.** Choline esterase distribution in fundus 84. Normal fed animal.

**Fig. 6.** Choline esterase distribution in pylorus 84. Normal fed animal.

*Experiments on Stomachs of Animals Injected with Atropine*

As in the case of acetyl choline, atropine was injected intramuscularly into fasting animals 30 minutes before they were killed; the dose
Fig. 7. Choline esterase distribution in pylorus 88. Same as used for data in Fig. 1.

Fig. 8. Choline esterase activity distribution in fundus 83. Animal injected with 100 mg. acetyl choline chloride ½ hour before killing.

used was an aqueous solution containing 200 mg. of atropine sulfate. At the time of killing, the effect of the drug was evident by the dryness in the mouth of the animal and the presence of relatively little
Fig. 9. Choline esterase distribution in pylorus 83. Animal same as used for data in Fig. 8.

Fig. 10. Choline esterase distribution in fundus 92. Animal injected with 200 mg. atropine sulfate ½ hour before killing.

fluid in the stomach; but the results presented in Figs. 10 and 11 show no real deviation from the normal picture.
Fig. 11. Choline esterase distribution in pylorus 92. Animal same as used for data in Fig. 10.

Fig. 12. Choline esterase distribution in fundus 91. Animal injected with 30 mg. eserine sulfate ½ hour before killing.

*Experiments on Stomachs of Animals Injected with Eserine*

Fasting pigs were given the injection intramuscularly as usual, and after 30 minutes were killed. The dose employed was 30 mg.
of eserine sulfate contained in aqueous solution. The reaction to
the drug at the time of killing was similar to that produced by 150 mg.
of acetyl choline chloride. Typical curves are given in Figs. 12
and 13. In this case as well, no marked change from the normal can
be observed.

A simple calculation enables one to estimate the quantity of acetyl
choline chloride split per cubic millimeter of tissue per second in the
various cell regions. Thus the epithelial cell portion of the tissue
yields activities of about 6 c.mm. N/20 HCl under the conditions
given; we may take 55γ as the corresponding approximate quantity
of acetyl choline chloride split (1 c.mm. N/20 HCl = 9.05γ). The
volume of two 25μ sections, 2.6 mm. in diameter, is 0.265 c.mm.

Hence $55\gamma \cdot \frac{1}{3600} \cdot \frac{1}{0.265} = 0.058\gamma$ split per second per cubic milli-
meter in pylorus, and $\frac{2}{3}$ this or 0.039γ in fundus and cardia.

**DISCUSSION**

The results presented all demonstrate the relatively intense choline
esterase activity associated with the epithelial cell portion of the
mucosa. Curves of methyl butyrase activity in the pig stomach (5) also showed relatively greater activity in the region of the greatest concentration of epithelial cells, but the magnitude of the enzyme hydrolysis was equivalent to 2.0–2.5 c.mm. N/20 HCl in 5 hours at 40°, while for choline esterase the corresponding activity was found to be 6.0–7.0 c.mm. N/20 HCl in 1.5 hours (1 hour in pylorus) at 30°. The pH (8.7) was a little above the optimum in the former, and a little below (8.0) in the latter case for the respective enzymes. The greater activity of choline esterase as compared to methyl butyrase was characteristic of all cells in the stomach.

No consistent profile was found in that portion of the curves beyond the epithelial cell region. In some samples of tissue the activity fell off and maintained a steady low value to the muscle layer, while in others a small increase in the locality of the chief cells was observed. Although it is not certain without detailed cell counts on each specimen, it would appear from the stained sections that the presence of epithelial cells in greater or lesser numbers is the basis of these variations.

The higher concentration of epithelial cells in the pylorus doubtlessly contributes much to the consistently greater choline esterase activity in this portion of the stomach. Crypts lined with these cells extend much deeper into the stomach wall in the pylorus than in the fundus or cardia regions. It is not uncommon to find epithelial cells close to the muscle layer. The possibility that the high choline esterase activity associated with the epithelial cell material may be the result of the presence of this enzyme in the interstitial tissue rather than in the cells, or in both, should not be overlooked.

It might be expected that choline esterase would be mobilized in response to the appearance of acetyl choline in the tissue. However, this does not appear to be the case since the injection of acetyl choline, in quantities sufficient to produce a reaction in the stomach, resulted in no observable change in the enzyme activity. From this, and the fact that the normal stomach in either the resting or actively digesting state shows no essential difference in choline esterase concentration, the suggestion would seem pertinent that a fairly constant concentration of the enzyme exists permanently in the tissue in question, and that this concentration is independent of such factors.
as nervous or direct chemical stimulation. Weight is lent to this
suggestion by the findings that the known inhibiting effect of eserine
upon the choline esterase in circulating blood (11-12) does not
apply apparently to the gastric mucosa under the conditions herein
described, no changes in enzyme activity of significance were elicited by
the eserine administered, and furthermore atropine gave no demon-
strable variation from the normal picture.

SUMMARY

Extraction experiments demonstrated that choline esterase could
be removed from microtome sections of tissue with as great facility by
0.9 per cent NaCl as by 30 per cent glycerol.

The quantitative distribution of choline esterase through the wall
of the pig stomach was studied, and it was found that the epithelial
cell region possessed the greatest activity and muscle tissue the
least. Pylorus was more active than fundus or cardia.

The enzyme activities found were independent of the physiological
state of the normal stomach at the time the animal was killed.

Neither intramuscular injection of acetyl choline, eserine, nor
atropine shortly before killing had significant influence upon the
activity in any region of the stomach.

The implications of these results were discussed.

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