GASTRIC CARBONIC ANHYDRASE IN DOGS

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Davenport and Fisher (1938) reported the discovery of large amounts of carbonic anhydrase in the gastric mucosa of cats, rats and rabbits. Davenport (1939) demonstrated that carbonic anhydrase is present in high concentration in the parietal cells and in lower concentration in the cells of the surface epithelium of the gastric mucosa of cats and rats. When those investigations were carried out dogs were not available.

At present work on the mechanism of the secretion of hydrochloric acid is being done using dogs as experimental animals. It is believed that carbon dioxide is formed in the parietal cells by the oxidation of a metabolite. This carbon dioxide is rapidly hydrated to carbonic acid with carbonic anhydrase catalysing the reaction. The carbonic acid then ionizes to give hydrogen ions and bicarbonate ions. By some means not at present understood the hydrogen ions are concentrated and secreted as the cations of hydrochloric acid while the bicarbonate ions are returned to the blood to replace the chloride ions removed in the acid secretion. Since the presence of carbonic anhydrase in the parietal cells is an important part of this hypothesis, it was thought desirable to demonstrate the occurrence of carbonic anhydrase in the gastric mucosa of dogs.

METHODS. The dogs were freed of blood by the viviperfusion method of Whipple (1926). The dogs were anesthetized with ether, and a cannula was inserted into the jugular vein. Ringer's solution containing 5 per cent glucose was injected by gravity, and the circulating fluid was allowed to flow out of the carotid artery at the same rate. By means of adrenalin injections the dogs' hearts were kept beating as long as possible. The hematocrit reading of the effluent fluid fell from about 38 per cent to below 1 per cent. At death the animals' tissues were not absolutely free of blood.

Carbonic anhydrase was determined by the method of Meldrum and Roughton (1933). The apparatus was used at 0°C. and at atmospheric pressure. The activity was calculated and expressed as enzyme units (E) according to the method of Meldrum and Roughton, but no correction was applied to bring the calculated activity to 15°C. The enzyme unit used in this work is therefore 2 to 3 times smaller than that of Meldrum and Roughton.
The distribution of carbonic anhydrase with respect to cell type was investigated by the method fully described by Davenport (1939). Cylinders 4.0 mm. in diameter were stamped from the gastric mucosa and placed on the table of a freezing microtome. The tissue was frozen, and sections 0.020 mm. in thickness were cut. Cutting was continued until three consecutive sections were obtained which were satisfactory. The 1st and 3rd slices were extracted with M/5 phosphate buffer, pH 6.8, and their carbonic anhydrase content was estimated.

The second slice was transferred to a microscope slide, fixed and stained. The slice was observed under a microscope, and the parietal cells in numerous fields taken over the whole slice were counted. Davenport (1939) has presented evidence to show that by this method a good estimate of the total number of parietal cells in the whole slice is obtained and that the number of cells found in the 2nd slice is equal to the mean of those in the 1st and 3rd slices.

RESULTS. Carbonic anhydrase was found in the extracts of the slices. The extracts catalysed the hydration and dehydration of carbon dioxide. The addition of the extracts to the phosphate buffer used in the enzyme estimation did not change the pH of the buffer as measured with a glass electrode. The end points of the catalysed and uncatalysed reactions were the same. The activity of the extracts was destroyed by 30 sec. boiling, by 30 min. at 65°C. and by 30 min. at pH 2 or 13. The activity was inhibited by M/800 HCN and by the specific inhibitor of Booth (1938).

The activity was probably not caused by the small amount of red blood cells remaining in the tissues. Dog red blood cells contain between 3.1 and 7.4 E per cmm. Up to 0.825 E was found in slices of the gastric mucosa having a volume of 0.25 cmm. If as much as 20 per cent of the volume of the slices consisted of perfusion fluid containing 1 per cent red blood cells the activity contributed by the carbonic anhydrase of the red blood cells would be only 0.0035 E per slice.

It was found that the slices fell into two distinct groups. The first group was composed of slices cut from the base of the glands, and they contained no surface cells. The second group was composed of slices cut from near the surface, and in addition to parietal cells and chief cells contained gastric pits made up of long tapering surface cells. The two groups will be considered separately.

For the first group the parietal cell count is plotted against the enzyme concentration in figure 1. The line drawn is the calculated regression line, and its equation is

$$Y = 0.000076 \times + 0.023$$

where Y is the enzyme concentration per slice and x is the number of parietal cells per slice. The correlation coefficient (r) calculated according
to the method of Fisher (1936) is +0.89, and there is less than one chance in a hundred that its true value lies outside the limits +0.54 to +0.98. It is unlikely that any other type of cell would have a nearly perfect correlation with the parietal cells over the whole range of from no cells to 9000 cells per slice which makes it equally unlikely that the correlation between the parietal cells and the enzyme concentration is an artifact. The intercept on the Y axis is negligibly different from zero, so it is improbable that in these slices there is any significant amount of carbonic anhydrase in any other type of cell. Consequently it can be concluded that carbonic anhydrase is confined to the parietal cells.

In the group containing surface cells the enzyme concentration was obviously not proportional to the number of parietal cells. The parietal cells were counted, and from the equation above the most probable amount of enzyme in that number of cells was calculated. This was subtracted from the total enzyme found. The surface cells were estimated, and their number is plotted in figure 2 against the residual enzyme concentration. The broken line is the parietal cell curve plotted on the same scale. Though the data are few and imperfect it can be concluded that there is probably a tenth as much carbonic anhydrase in the surface cells as in the parietal cells.
Conclusions. It has now been shown that there is a high concentration of carbonic anhydrase in the parietal cells of cats, rats and dogs. Since carbonic anhydrase has also been found in the gastric mucosa of several other mammals it is likely that parietal cells in all species contain carbonic anhydrase.

On account of such high concentration of carbonic anhydrase in the parietal cells the equilibrium between carbon dioxide and carbonic acid must be reached with extreme rapidity. The secretion of hydrochloric acid is doubtless a dynamic process, and during activity equilibrium is probably never quite attained. The observation of Bulger, Allen and Harrison (1928) that during acid secretion the bicarbonate content of the gastric venous blood rises leads to the conclusion that during activity carbon dioxide is continuously being produced within the cells and hydrated to carbonic acid and that the bicarbonate ions formed after the ionization of the carbonic acid are steadily flowing into the venous blood. The uncatalysed hydration of carbon dioxide is relatively slow, and if carbonic anhydrase were not present that reaction would be the limiting one in the chain. In fact the carbon dioxide would not be hydrated within the cells but would diffuse as such into the red blood cells where under the influence of the carbonic anhydrase present in the red blood cells it would be hydrated. Therefore the hydrogen ions produced by the subsequent ionization of the carbonic acid would be lost to the parietal cells and would instead be absorbed by the buffering mechanism of the blood.

The hypothesis that carbonic anhydrase occupies a central position in the mechanism of the formation and secretion of acid is further supported by unpublished observations that when carbonic anhydrase in the gastric mucosa is inhibited the secretion of acid is likewise inhibited to exactly the same degree as that to be expected if the rate of secretion is directly proportional to the rate of hydration of carbon dioxide. This work will be the subject of a paper in preparation.

Summary

Carbonic anhydrase is present in high concentration in the parietal cells and in lower concentration in the cells of the surface epithelium of the gastric mucosa of the dog. It is probably not present in any other type of cell in the gastric mucosa.

References

Whipple, G. H. This Journal 76: 693, 1926.