New Observations on the Alkaline Glycerophosphatase Reaction in the Papilla Foliata.*

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The histochemical demonstration of alkaline glycerophosphatase in the papilla foliata of the rabbit's tongue was reported by Bourne (3) and by Baradi and Bourne (1). The aim of the present investigation was to record the speed of the histochemical reaction and the detailed distribution of the enzyme in this tissue. Fixation was carried out in the ice box using 85 per cent alcohol for 3 hours followed by absolute alcohol for another 3 hours. Sections were incubated in the Gomori substrate mixture for alkaline glycerophosphatase (6), and the cobalt-sulphide method was used for visualizing the calcium phosphate precipitated at the sites of the enzyme activity.

After only 1 second's incubation, a very faint reaction was obtained, but it was visible only on careful microscopical examination. It was localized in the most superficial layers of the epithelial lining of the upper ⅔ of the lateral walls of the gutters separating two individual papillae. A reaction in the same area was obtained after 5 and 15 seconds' incubation (Fig. 2) but in both cases it was greatly intensified compared with the 1 second preparation, and could be seen with the naked eye. In these three preparations the reaction was obviously located between the cells and could have been in the cell membranes or in the intercellular substance, or both. After 45 seconds' incubation the reaction extended into the deeper layers of the epithelium to include those cells that directly overlie the taste buds (Fig. 3). In these deep layers of cells, in addition to a reaction which outlined the cells (Fig. 4 *a*), there was a peripheral granular cytoplasmic staining (Fig. 4 *b*). After 2 minutes' incubation the reaction extended down to the cells lining the bases (lower ⅓) of the gutters where it followed the outlines of the cells (Fig. 5). Here also, there was a peripheral granular cytoplasmic staining. Increasing the incubation time to 45 minutes increased only the intensity of the staining with the distribution of the enzyme the same as with 2 minutes. After 1 hour (Fig. 6) the reaction appeared in all the sites previously known from the literature to be positive (1). In addition to the epithelial lining of the gutters there was another site of activity that is in the subepithelial connective tissue corium where there was a fairly strong reaction of a fibrillar nature that seemed to be associated with the fibres of the subepithelial nerve plexus which runs in that area (2, 7). Taste buds remained negative. The enzymatic picture obtained after 2, 4, and 25 hours' incubation was the same as that obtained after 1 hour but was more intense. It can be assumed therefore that diffusion artefacts played no significant part in the results here described.

A surprising observation in the present investigation is the fact that an alkaline glycerophosphatase reaction was obtained histochemically in the papilla foliata after 1 second's incubation in Gomori substrate mixture. However the authors were also able to obtain a reaction in the brush border of the intestinal epithelium of the rabbit after 1 second's incubation and in the brush border of the kidney convoluted tubule cells after 5 seconds' incubation. Therefore the enzyme activity of the papilla foliata is comparable with that of the brush borders of intestinal and kidney tubule cells (Figs. 1 *a* and *b*, Fig. 2), the classic and probably the most intense hitherto recorded sites of alkaline glycerophosphatase activity. It is worth mentioning here also that the full enzymatic picture obtained in the previous work (1) in the papilla foliata after 3 to 4 hours' incubation using a Gomori substrate mixture with calcium ion concentration of ±0.02 M, has been obtained in the present investigation after 1 hour using ±0.10 M calcium ion concentration.

One extremely interesting aspect of this work is that it demonstrates a relationship between nerve fibres and phosphatase in the gustatory epithelium which is similar to that previously demonstrated in the olfactory mucosa (1, 2). In these two tissues a similar arrangement in which processes of neurons known to be rich in cholinesterase are closely invested by phosphatase-active material has been observed (Fig. 7). In the papilla foliata, Baradi and Bourne demon-

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strated cholinesterase in the nerve fibres in the epithelium of the lateral walls of the gutter (2) and the present work has shown that the environment in which these nerve fibres run is rich in phosphatase which may be localized either in the intercellular substance or in the nearby cell membranes. In the olfactory mucosa, the axons of the olfactory cells are known to be very closely enveloped by loops of the plasma membrane of the basal cells as they pass to the fila olfactoria (5). Baradi and Bourne have shown that these axons contain true cholinesterase (2) and that the basal cells are extremely rich in phosphatase (1). The role of phosphatase in these sites in relation to impulse propagation can only be surmised, but one must consider the possible relationship of the enzyme to cell membrane penetration (8) and the previously expressed views of the present authors (1) which were that the enzymatic activities in the neighbourhood of the nerve fibres in the gustatory epithelium resulted in depolarization and the production of an action potential. It is now planned to study the distribution of the phosphatase reaction in these sites by electron microscopy using the technique of Brandes *et al.* (4) and so to determine the position of the enzyme in relation to the cell membrane.

References

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Explanation of Plate 84

Fig. 1. Alkaline glycerophosphatase in the brush borders of the kidney convoluted tubule cells of the rabbit (1 *a*) and in the brush borders of the intestinal epithelium of the rabbit (1 *b*) after 5 seconds' incubation in Gomori's substrate mixture. In the former preparation blood cells inside the blood vessels show a faint reaction. × 400.

Fig. 2. Alkaline glycerophosphatase in the papilla foliata of the rabbit after 5 seconds' incubation in Gomori's substrate mixture. The superficial cells (*S*) of the epithelial lining of the upper ⅔ of the lateral walls of the gutter (*G*) separating two papillae (*P*) stained fairly strongly. × 215.

Fig. 3. In the papilla foliata, after 45 seconds' incubation, all the cells of the lateral walls of the upper ⅔ of the gutter showing a fairly strong reaction. × 215.

Fig. 4. Same as Fig. 3, but under oil immersion objective. Notice staining of cell outlines (4 *a*) (See arrow) and peripheral granular cytoplasmic staining (4 *b*) (See arrow) in the deep layers of cells that directly overlie the taste buds (*T*).

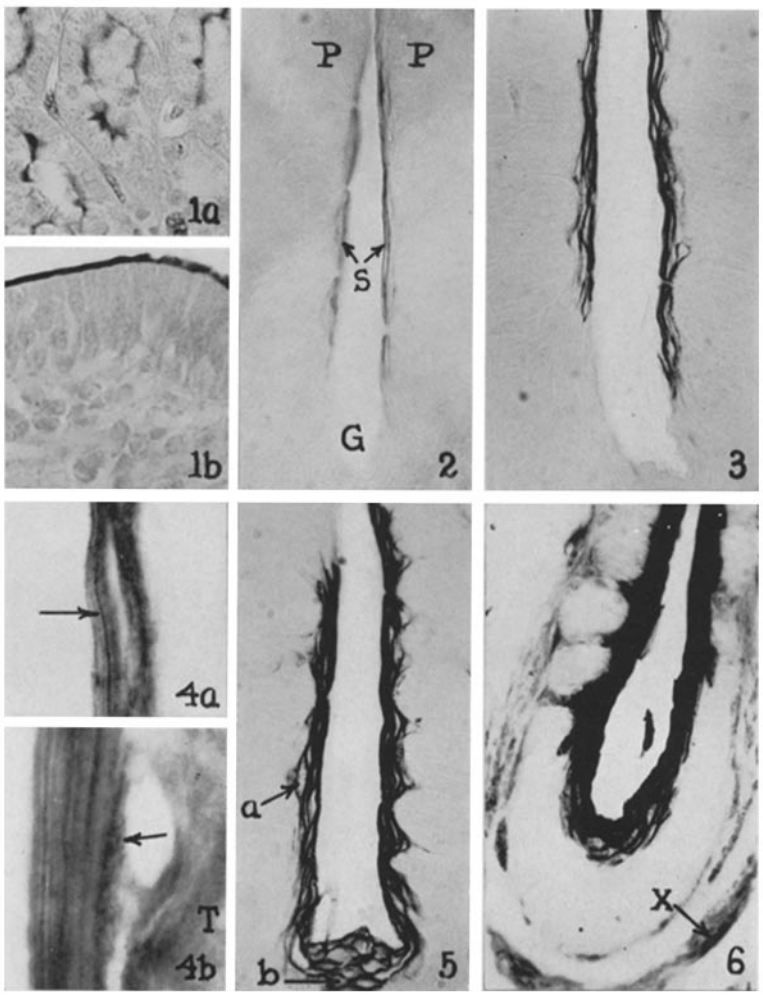
Fig. 4 *a*, × 1245.

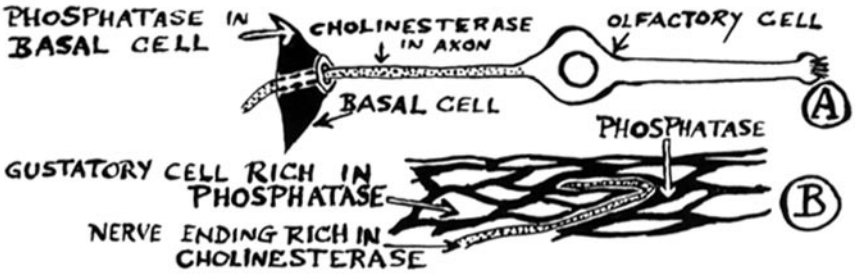
Fig. 4 *b*, × 2490.

Fig. 5. After 5 minutes, all the cells lining the gutter were stained. Cell outline staining can be seen in two sites *a* and *b*. × 215.

Fig. 6. After 1 hour, in addition to the cells lining the gutter, the subepithelial nerve plexus (*X*) also stained. × 215.

Fig. 7. Diagrammatic representation of olfactory cell axon (*A*) and nerve ending in gustatory epithelium (*B*). The close relationship between the choline-esterase–positive nerve fibres and phosphatase-positive surrounding cells can be seen.





(Baradi and Bourne: Alkaline glycerophosphatase reaction)