Department of Anatomy (Prof. T. WASANO), Faculty of Medicine, 
Kyushu University, Fukuoka, Japan

Histochemical Observation on the Phosphatases of the Tongue, 
with Special Reference to Taste Buds

Takashi IWAYAMA and Osami NADA

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The sensory receptor for taste is generally considered to be the taste bud, which in mammals is found mainly in association with the papillae of the tongue. Although the receptor potential of a single taste cell in the rat has been recorded by Kimura and Beidler (1961), the kind of change or changes occurring in the taste cell initiating this receptor potential is still unknown. Recent electron microscopic observations (Trujillo-Cenoz 1957, de Lorenzo 1958, 1963, Murray and Murray 1960, Nemetschek-Gansler and Ferner 1963, Farbman 1965, Gray and Watkins 1965, Hirata 1966) have greatly advanced and enlarged the knowledge concerning the structural details of the taste organ, but have afforded no specific clues that might serve to resolve this problem. What role enzymes play in the mechanism of taste is still uncertain, since electro-physiological experiments heretofore conducted have not resulted in equivalent or even similar conclusions (Beidler 1955, Yureva 1957, 1961, Koshtoiants and Katalin 1958).

Several enzymes have been histochemically demonstrated in the gustatory region, namely, alkaline phosphatase (Bourne 1948, Baradi and Bourne 1951, 1953, 1959), succinic dehydrogenase (Rakhawy 1962a) and cholinesterase (Baradi and Bourne 1959, Ellis 1959). Alkaline phosphatase activity, which is found in the superficial layers of the epithelium overlying the taste buds, was presumed to play a significant role in the physiology of taste according to Baradi and Bourne (1951) and Rakhawy (1962b). It is not known whether these assumptions are true or not, but it would be particularly important to examine enzyme distributions in order to correlate any possible morphology and function.

To this end, the following histochemical experiments were performed, using rabbits and rats, to determine the presence of alkaline phosphatase, adenosine triphosphatase and acid phosphatase of the tongue.

Materials and Methods

Adult albino rats and rabbits were anesthetized with ether. The tongues were then removed including the foliate, fungiform and circumvallate papillae. The specimens were immediately frozen on solid carbon dioxide, following trimming in order to make subsequent suitable sections of each papilla. The frozen sections were then cut in a cryostat in 15 μ thicknesses, dried at room temperature and processed in an incubation mixture for the determination of following enzymes,
For detection of alkaline phosphatase (alk. Pase) activity, naphthol AS method of Burstone (1961) was employed using naphthol AS-MX phosphate and Fast Blue RR salt. Incubation was carried out at room temperature for 3 to 15 min.

For the determination of adenosine triphosphatase (ATPase) activity, the sections were incubated after the method of Wachstein and Meisel (1957) for 10 to 20 min. at room temperature substituting 0.2 M cacodylate buffer for 0.2 M tris buffer. The sections were then developed in dilute yellow ammonium sulfide after thorough washing in three changes of distilled water.

In demonstrating acid phosphatase (acid Pase) activity, the naphthol AS method by Goldberg and Barksa (1962) was adopted, applying naphthol AS-BI phosphate as the substrate, and incubating for 40 to 60 min. at 37°C. The histochemical controls used were composed of L-cysteine (10^{-3}M), PCMB (2.5 \times 10^{-3}M) and sodium fluoride (10^{-2}M) for alk. Pase, ATPase and acid Pase, respectively. All the sections were first immersed in their respective inhibitor solutions and then incubated in the previously described admixture with inhibitor.

After the incubation, the sections prepared for acid and alk. Pase were mounted in 5 per cent glycerin jelly, while, the sections for ATPase were first dehydrated by passing through an ascending series of alcohol solutions, then in xylol and mounted with Canada balsam.

**Observations**

The observations on both species examined, although essentially similar, differed in a few respects. The following descriptions were taken mainly from rats, except where otherwise stated.

*Fig. 1.* Alk. Pase of a rat's circumvallate papilla. The activity is localized on the superficial layers of the epithelium lining the gutters. The capillary wall in the lamina propria shows considerable activity. 10 min. incubation. × 120
Alkaline Phosphatase

In both the circumvallate and foliate papillae, the activity was found on the superficial layers of the epithelium lining the gutters which are studded with taste buds (Figs. 1, 3), thus confirming the findings of Bourne (1948). The epithelium surrounding the outer taste pores was the first site to react, the reaction thereafter extending along the superficial layers. No activity was found on the epithelium overlying the free surfaces of both foliate and circumvallate papillae, nor on those of the fungiform papillae, where a taste bud is usually located (Fig. 2). Taste bud cells had, in general, no activity but occasional cells did have a reaction in their apical halves, which possibly was a diffusion artifact arising from the strongly reactive epithelium (Fig. 1).

Alk. Pase activity was also present in the lamina propria where capillary walls were responsible for the reaction (Fig. 1). Sensory nerve fibers and autonomies with associated groups of ganglion cells in the lamina propria of circumvallate papillae can be seen but the ganglion cells are indistinct at the base of foliate papillae. These nervous elements were but slightly reactive for the enzyme. The salivary glands, both serous and mucous secreting, which are embedded deeply in the muscular layer, presented an intense activity at the basal portion of the acinar cells (Fig. 5). No such observations were noted in rabbits: the ganglion cells and their nerve fibers had the strongest activity (Fig. 4), while the sensory nerve fibers had the next most strong (Fig. 3): the salivary glands demonstrated only a very faint activity. Capillary walls showed only an occasional weak reaction.

L-cysteine at a concentration of 10⁻³M. inhibited all activity of alk. Pase.

Adenosine Triphosphatase

The taste bud cells were clearly demonstrated in the non-reactive stratified
Fig. 3. Alk. Pase of a rabbit's foliate papilla. The activity is similar to that of the rat's foliate papilla in the epithelium, but differs in the lamina propria: i.e., the reaction is evidenced along subgemmal nerve fibers but minimally on the capillary wall. 10 min. incubation. × 100

Fig. 4. Alk. Pase of a rabbit's ganglion cells located near a salivary gland. Muscle can be seen at the upper right and salivary gland, at the lower left. Both show no reaction. 15 min. incubation. × 450

Fig. 5. Alk. Pase of a rat's salivary gland. Intense activity is observed on the basal portion of acinar cells, presumably on myoepithelial cells. Acinar cytoplasm includes granular deposits. 8 min. incubation. × 150
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The squamous epithelium covering the papillae, following the reaction for ATPase activity (Figs. 6, 7). The reaction product seemed to be associated almost entirely with the membrane of the taste bud cells, appearing first and most intensely on the so-called taste hairs (Fig. 7). Also, minute granular deposits were noted throughout the cytoplasm of the taste bud cells. Careful examination of the stratified epithelium revealed some intraepithelial nerve endings with activity. The nerve fibers just under the epithelium which are running in parallel with the gutters, as well as larger nerve fibers found at the base of papillae, gave a definitely positive reaction for the enzyme (Figs. 6, 7). The ganglion cells located in the core of the circumvallate papilla and between the salivary glands were also strongly reactive (Figs. 6, 8).

As for blood vessel reactivity, both the capillary walls and the smooth muscle of the lingual artery were specifically reactive for the enzyme. Intense activity was also observed along the delicate nerve fibers wrapping the artery (Fig. 9).

The localization of ATPase activity within the salivary glands differed in both species, that is, the activity was found at the periphery of acinar cells in rats (Fig. 10), but on the membrane lining of the glandular cavity in rabbits (Fig. 11). All the sections, previously immersed in PCMB at a concentration of $2.5 \times 10^{-3}$M revealed no reaction at all.

**Acid Phosphatase**

This activity was seen to be more intense in the taste bud cells than in the other epithelial cells which showed only a diffuse pinkish coloration (Fig. 12). The reaction was localized to the supranuclear cytoplasm of the taste bud cells in which granular deposits were observed against an uniformly stained background (Fig. 13). Although
Fig. 7. ATPase of a rabbit's foliate papilla. The activity seems to be mainly in association with the membrane of taste bud cells. The most intense activity can be seen on the so-called taste hairs. 20 min incubation. × 350

Fig. 8. The ganglion cell, appearing in the core of a rat's circumvallate papilla, exhibits strong ATPase activity. The nucleus has no reaction. 15 min incubation. × 680

Fig. 9. A tangential section of arteries supplying the lingual muscle. The delicate autonomic nerve fibers wrapping the arteries show ATPase activity. 15 min incubation. × 230
the taste bud cells reacted either intensely or weakly, some difficulty was encountered in differentiating the two kinds of cell types, neuroepithelial and sustentacular.

Many fibroblasts with the intense activity can be seen in the lamina propria

Fig. 10. The salivary gland, located deeply in the muscular tissue of the posterior region of a rat's tongue, demonstrates ATPase activity on the basal portion of the acinar cells. The serous part is seen on the right in the bottom of the figure. 12 min. incubation. × 130

Fig. 11. The salivary gland of a rabbit's tongue. ATPase activity is seen mainly on the membrane lining the glandular cavity. 15 min. incubation. × 350
The ganglion cells also showed an intense activity but the nerve fibers themselves stained faintly (Fig. 14). Capillaries gave no reaction whatsoever.

(Fig. 12). The ganglion cells also showed an intense activity but the nerve fibers themselves stained faintly (Fig. 14). Capillaries gave no reaction whatsoever.
Acid Pase of the salivary glands appeared in the cytoplasm of the acinar cells, however, it was not evident within mucous glands because the cytoplasm of mucous cells is projected upon the basal portion causing subsequent displacement of the gland by the accumulation of mucigen (Fig. 15). The excretory ducts stained conspicuously. Acid Pase activity was completely suppressed by $10^{-2}$M of sodium fluoride.

**Fig. 15.** Acid Pase of a rat's salivary gland. The activity is localized in the cytoplasm of the acinar cells. A mucous gland is at the upper left and a serous gland at the lower right. 50 min. incubation. $\times 260$

**Discussion**

The present experiments demonstrated that phosphatase activities have special localizations relative to taste bud cells. As shown first by BOURNE (1948), alk. Pase is found primarily in association with the superficial layers of the epithelium covering the gutters of both foliate and circumvallate papillae. Later, BARADI and BOURNE (1951, 1953) examined histochemically the effect of various substances with taste producing properties upon alk. Pase and hypothesized that the interference of this enzyme with these substances caused the taste sensation, but RAKHAWY (1962b) assumed, on the other hand, alk. Pase in this region played an important role for the transport of the gustatory substances into the taste pores. Although these assumptions are of interest and should be regarded, special consideration must attend the fact that the epithelium covering fungiform papillae, on which a taste bud is located, has no alk. Pase activity. The superficial cells of the stratified epithelium are continuously undergoing degeneration with subsequent sloughing. It should be worthy of note that in such a portion there is strong enzymatic activity. There have been many experiments concerning the effect produced by transection of the innervating nerve of the taste bud. These have consistently demonstrated that the taste buds disappear after severance of their nerve fibers (TORREY 1934, 1940, GUTH 1957, 1958).
It seems, therefore, of interest to pursue the change of alk. Pase activity which is to be found, not on the taste buds themselves, but on the epithelium overlying them. Indeed, the specimen in which the nerve had been severed in advance showed clear decrease of alk. Pase activity. These results will be published in a subsequent report.

The observations on both rats and rabbits were, as described above, generally similar but differed in a few respects, especially in alk. Pase activity. In rabbits, nerve cells and fibers in the lamina propria demonstrated a considerable amount of alk. Pase, whereas it was only slightly recognized in the capillaries. In rats, on the other hand, the reverse was true. The capillaries showed a strong activity while nerve cells and fibers only a very weak. Alk. Pase and ATPase have been demonstrated in the basket cells of rat's salivary glands (Shear 1964). In this experiment, the glandular cells in rats demonstrated both alk. Pase and ATPase in their basal parts but none was seen in rabbits. These differences were quite unexpected and will warrant further examination.

ATPase activity was observed most specifically in the taste bud cells with the three kinds of phosphatase investigated. The membrane of taste bud cells would seem to be the actual enzymatic site, although no definitive conclusion could be made on examination with the light microscope. It is hardly plausible that this histochemically demonstrable ATPase is the membrane ATPase being considered but the latter is deemed easily converted from the other types of ATPase. Recent biophysiological data have stressed the importance of the membrane ATPase for active transport of ions through the membrane (Skou 1965). However, Yur'eva (1957, 1961), following electro-physiological experiments using SH blocker and SH donor, suggested the possibility that the sulphhydril groups of proteins participated in the process of taste perception. In the present experiments, ATPase was completely inactivated with PCMB which is a selective inhibitor to the SH group. These results might suggest that the ATPase demonstrated here might be functionally associated with the membrane ATPase and might serve for the reestablishment of ionic concentrations after receptor potentials (Kimura and Beidler 1961) have been discharged. Recent biochemical and histochemical research has shown the heterogeneity of ATPase (Hori and Chang 1963) and, therefore, differentiation and identification of this ATPase found in these experiments remains to be determined.

There were also granular deposits throughout the cytoplasm of the taste bud cells. These might be mitochondrial ATPase (Otero-Vilardebo et al. 1963, Goldfischer et al. 1964), although the possibility that these are diffusion artifacts from the strong reactive sites such as the cell membrane and subgemmal nerve plexus must also be considered. Intense ATPase activity was found on sensory and autonomic nerve fibers in the lamina propria, as shown by Mustakallio (1962) in human epidermis, but this activity seemed to be associated mainly with the Schwann cells, particularly of the large nerve fibers. Ellis (1959) reported that numerous nerves of varying size in the arteries and arterioles supplying the lingual muscle demonstrated strong cholinesterase activity. Autonomic nerve fibers seem to have strong ATPase activity as well (Fig. 9).

There is continuing controversy regarding the nature and type of cells found within the taste bud cells. Farbman (1965) differentiated four cell types and observed the existence of a lamellated dense body similar to a lysosome in the type I cell (taste
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cell). This evidence might indicate simply a variation in taste bud cell due to staining with acid Pase. Moreover, electron microscopic cytochemistry has recently revealed that there is acid Pase activity associated with the Golgi apparatus and secretory granules (SOBEL and AVRIN 1965, LAZARUS et al. 1966). Intense acid Pase activity in the supranuclear portion of taste bud cells might indicate a possible secretory function for these cells.

Summary

Alkaline phophatase, adenosine triphosphatase and acid phosphatase activity was demonstrated in the tongues of the rat and rabbit, especially in relationship to taste buds.

1. Alkaline phosphatase was localized on the superficial layers of the epithelium of the taste buds in the circumvallate and foliate papillae, as reported, but no activity was found on the superior surface of the epithelium of the fungiform papilla. The capillary walls of rat, and the ganglion cells and nerve fibers of rabbit were reactive for the enzyme. The acinar cells of the salivary gland in the rat demonstrated a positive activity on their basal portion but this was not so in the rabbit.

2. ATPase was found to be specific in taste bud cells on the epithelium covering the papillae. Its activity seemed to be almost entirely associated with the membrane. In the lamina propria, a strong activity was evidenced in ganglion cells, nerve fibers and the walls of blood vessels. The activity of salivary gland was found at the basal portion of acinar cells in rats, while on the membrane lining the glandular cavity in rabbits.

3. Acid phosphatase activity was intense in the taste bud cells, especially in the supranuclear region. In the lamina propria, fibroblasts and ganglion cells were responsible for the enzyme. Nerve fibers were essentially unreactive. Salivary glands and their excretory ducts demonstrated activity in the cytoplasm of their respective cells.

The possible functions, regarding these enzymes, have been discussed.
るか、ウサギにおいては腺細胞の腺腔に面する側に活性を有する。
酸フォスファターゼ活性は上皮においては味蕾細胞にとくに強く、しかも細胞の核上部に限局して見られた。活性はすべての細胞に同じように現われるのではなく、強いものと弱いものがある。
粘膜固有層では線維芽細胞や神経細胞には活性が見られるが、神経線維にはほとんど活性を見ない。
唾液腺においては活性は腺細胞の胞体に見られるほか、腺導管部も活性を有する。
これら酵素の意義についても、その可能性について討論を加えた。

References

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