THE INFLUENCE OF NERVE FIBERS UPON TASTE BUDS DURING EMBRYONIC DEVELOPMENT

BY THEODORE W. TORREY

DEPARTMENT OF ZOOLOGY, INDIANA UNIVERSITY

Communicated September 30, 1940

It has long since been demonstrated in a variety of animal forms that subsequent to experimentally induced degeneration of gustatory nerve fibers the taste buds associated with them disappear. It has also been shown that when the nerve fibers regenerate, new buds make their appearance. Taste buds, therefore, are intimately dependent for their existence upon their nervous supply; and by way of explaining the possible physiological nature of this dependence, a neurohumoral mediator has been hypothesized (Torrey, '34a, '36).

On the basis of accounts given by Hermann ('84), Gräberg ('98) and others, it has been inferred that nerves probably play a similar rôle in the ontogeny of gustatory organs. Hermann, for instance, maintained that nerve fibers came into contact with the epithelium of the developing papillae on the tongue of the foetal rabbit at the same time the anlagen of the taste buds were appearing, and thus nerves were presumed to have stimulated or induced the formation of the sense organs. Szymonowicz ('95, '96) made a similar suggestion for the Corpuscles of Grandry and Herbst, and more recently Whiteside ('26), speaking of the regeneration of taste buds in the rat, has said that inasmuch "as the regenerative process resembles the embryonic, the causative factor would probably be the same in both cases."

Other workers, by contrast, have not accepted this inference. Herbst ('01) and Harrison ('04), for example, have said that the coincidental appearance of nerve fiber and sense organ is not proof of the relation of cause and effect. Harrison has gone further and like Roux differentiated between a "function of development" and a "function of conservation;" that is, the power to differentiate is inherent within the anlage itself, whereas the stimulus that subsequently maintains the organ is supplied by the nerve.
This conception is furthered by Patzelt ('24) who maintains that not only is the ability to differentiate into taste buds inherent within the epithelium, but that the nerve fibers actually grow in secondarily. And finally, Stone ('33, '40) concludes from his studies of grafted tongue primordia in Amblystoma that in this form, at least, nerves do not play a causative rôle in the formation of taste buds.

Obviously the intimate dependence of taste buds upon their nerve supply exhibited during degeneration and regeneration in the adult individual has not been demonstrated to exist for certain during ontogeny. An attempt has been made, therefore, to solve this long-time problem. The investigation has followed two courses. The first consisted of working out the normal embryogeny of the sense organs and their nerve fibers. This was necessarily prerequisite to the second: an experimental analysis of the morphogenetic association prevailing between the two. All experiments and observations have been performed on the rat and attention directed solely towards the vallate papilla of which there is a single one only in the rat.

The development of the vallate papilla (as of all the other papillae) precedes that of the taste buds by a considerable time. The single papilla originates in a manner so similar to that already described for those of the rabbit (Hermann, '84) and man (Gräberg, '98), that additional description is hardly warranted. Suffice it to say for the rat that the papilla begins to take form towards the end of the fifteenth day of gestation and is completely established by the third day postpartum. The first taste buds, however, do not appear until about the ninth day after birth. This cannot be said to represent an absolute date, for some individual variation occurs. In one or two instances, for example, an occasional bud was to be seen early on the eighth day, whereas on other occasions buds could not be detected until the tenth. Generally speaking, however, the time of initial appearance of the buds is the ninth day. Few in number at this time, they gradually multiply, reaching a maximum in about twelve weeks, from which time they undergo a slight reduction in numbers until an adult constant of roughly 75 to 100 buds, associated exclusively with ventral levels of the papilla and bounding trench, is attained.

Special attention was directed towards the time of arrival of the ingrowing nerve fibers at their epithelial terminals. For this purpose whole embryos and pieces of tongues ranging from a prenatal age of fifteen days to twelve days postpartum were fixed in formol-acetic-alcohol and stained with activated protargol (Bodian, '37).

Other workers have shown that the single anlage of the glossopharyngeal ganglia is established towards the beginning of the twelfth day of gestation and promptly divides into a dorsal group of cells, the future superior ganglion, and a ventral group, the future petrosal ganglion. Processes are soon
given off from the neuroblasts and by the end of the thirteenth day a definitive glossopharyngeal nerve has connected centrally with the brain and is pushing peripherally towards its final terminations. My own observations reveal that as soon thereafter as the beginning of the sixteenth day a fairly conspicuous bundle of fibers is present in the basal portions of the developing tongue lying not far beneath the dorsal epithelium. These fibers continue peripherally during the succeeding two days, reaching and even extending beyond the level of the already developing vallate papilla. Sections of a twenty-day tongue reveal nerve fibers which have entered and traversed the subepithelial tissues of the papilla to terminate in the form of a subepithelial plexus. From this time until the first taste buds appear some ten days later the number of fibers within the papilla, and thus at the same time the extent of the subepithelial plexus, are increased by the arrival of new bundles of fibers from various directions beyond the papilla.

A few of the preparations of postpartum stages previous to the ninth day show occasional fibers which have penetrated the papillary epithelium. Such intraepithelial fibers are so difficult to demonstrate histologically that an accurate count of their absolute number has not been attempted. Sufficient of them are stained, however, to demonstrate unquestionably that such fibers are present several days in advance of taste bud differentiation.

If one is committed to the proposition that nerves do incite or determine the formation of taste buds, then the above facts lend themselves in support. The precocious arrival of the fibrils at the site of the future end organs may, however, be entirely without significance other than that a nervous supply is ready and waiting for the buds when they do appear, for it is yet to be demonstrated that the fibers literally exert a "stimulus of development" which brings the end organs into existence. A critical test would consist in setting up conditions whereby the buds would be permitted, if they are capable of doing so, to develop free from the influence of nervous elements. This has been attempted in two entirely different fashions: first, by causing a previously destroyed papilla in the adult to regenerate in the absence of functional nerve fibers; second, by transplantation of papillae of pre-taste bud ages.

Whiteside ('26) has shown that following complete extirpation, the vallate papilla of the rat begins to regenerate within three weeks and in six months is completely restored. New taste buds likewise develop in the epithelium of the regenerating papilla, the first buds usually appearing about the same time the papilla proper begins to take form, i.e., at three weeks, and continuing to be formed for as long as twenty-two weeks.

In the light of the known relations of gustatory organs to degenerating and regenerating nerves, buds are formed out of the newly established epithelium of a regenerating papilla presumably under the influence of the
gustatory fibers likewise resupplying the papilla. Assuming that the regeneration process is closely akin to the original ontogeny, a test of the taste bud forming capabilities of a regenerating papilla devoid of functional nerves might be expected to give a partial answer to the question of the ontogenetic relation of the sense organ and nervous supply. The conditions for such a test have been set up by destroying the vallate papilla and cutting the glossopharyngeal nerves which supply it.

Four to seven months old male and female albinos were used for most of these experiments, supplemented by a few individuals from a chocolate hooded stock employed for the transplantation experiments to be described later. Individuals were anesthetized with sodium amytal and the vallate papilla cauterized. Because most of the buds have a bilateral innervation (Whiteside, '27) it was necessary to cut both glossopharyngeal nerves. Each was located where it lay dorsal to the anterior belly of the digastricus muscle and severed proximal to the junction of its pharyngeal and lingual branches.

Fifteen animals were operated upon in this fashion. For controls fifteen animals with the papillae alone destroyed were used. Three experimental and three control animals were sacrificed two weeks after operation and the tongues prepared for study. Additional groups of three each were sacrificed so as to comprise a series with the groups separated by four-day intervals. There was thus available material of fourteen, eighteen, twenty-two, twenty-six and thirty days post-operative ages.

At the fourteen-day stage in both the experimental and control animals, the regeneration process has gone little beyond the wound healing stage. The papilla has not yet begun to reform and there are no taste buds present. Four days later there are evidences of the epithelial proliferation preceding the formation of a definite papilla. The papilla gradually begins to take form during the succeeding stages in both the experimental and control animals. The details of its regeneration conform to those already given by Whiteside. The one additional contribution made by the present investigation is that regeneration of the papilla goes on independent of the nervous system; the known dependence of regenerating taste buds on nerve fibers does not prevail for the papilla. It is interesting to note in this connection that the barbels of the catfish, by contrast, will not regenerate in the absence of a normal nerve (Olmsted, '20).

As far as the buds themselves are concerned, the first newly formed ones were observed in the control animals of twenty-six days. There were three buds in one of these, and five in the other two. The three controls of the thirty-day stage showed twelve buds in one, thirteen in the second and fifteen in the third. These observations on the number and time of first appearance of the buds conform fairly closely to those recorded by Whiteside.
In the experimental animals, by contrast, buds were observed in only one instance, that of one of the three thirty-day individuals, and in this case two well-defined buds were seen. There was reason to believe, however, that regenerating nerve fibers from the central trunk may have reached the papilla and induced the buds to form out of the regenerating papillary epithelium. To test this likelihood the papillae of four animals were cauterized at one time and the nerves severed ten days later. This was considered an interval sufficient to prevent the normal nerves from exerting any influence upon the reforming papilla while at the same time shortening the period available for the return of the new fibers. Two of the animals were sacrificed thirty days after cauterization and two at thirty-five days. There were no taste buds present in any of the four.

The conclusion to be derived from these experiments, then, is that in the adult individual taste organs regenerate only under the influence of nerve fibers. To carry this conclusion over to the embryonic origin of the buds, however, requires the assumption that the regeneration of an organ is strictly comparable to its ontogeny, an assumption not wholly justified by the comparative data of regeneration phenomena in general. It remains to be demonstrated that this "function of conservation" on the part of nerves exists as a "function of development." The following experiments comprise an attempt at such a demonstration.

A crucial test of the ability of the developing tongue to give rise to taste buds in the absence of nervous stimulation involves setting up conditions whereby embryonic differentiation of the papilla would continue in an environment totally devoid of nervous elements. It was believed these conditions would be found in some variety of graft.

Preliminary experiments showed that subcutaneous, omental and ear grafts were unsuitable for several reasons. Not only did the transplanted tissues degenerate rather rapidly, but were subject to distorting pressures and were commonly invaded by considerable amounts of fat and connective tissue. There was the ever-present possibility, too, that such grafts, even if they survived and continued differentiation, would be invaded by nerve fibers from the surrounding host tissues. This eventuality alone would defeat the purpose of the experiment. It was necessary, then, to employ an environment of a more "neutral" character. To this end resort was had to the anterior chamber of the eye, a site offering many of the advantages of explanation methods and few of the technical difficulties.

The material, host and donor, for such grafts was derived from a strain of chocolate hooded rats inbred for 25, 26 and 27 generations. The original 25th generation stock was obtained through the courtesy of Dr. R. T. Hill of the Indiana University Medical School. Later in the project this material was supplemented by Sprague-Dawley albino hosts and donors which proved to be equally satisfactory. The transplants were obtained from
donors ranging from a foetal age of sixteen days to eight days postpartum (the interval during which the vallate papilla and its nervous supply develop, but terminating just short of the time of the initial appearance of taste buds). The age of the host animals ranged from 120 to 212 days.

The accompanying table summarizes the history of the grafts.

<table>
<thead>
<tr>
<th>EXPT. NO.</th>
<th>NO. OF RECOVERED GRAFTS</th>
<th>DONOR</th>
<th>AGE OF GRAFT (DAYS AFTER OPER.)</th>
<th>THEORETICAL POST-PARTUM AGE</th>
<th>AGE BEYOND NORMAL TASTE BUD THRESHOLD</th>
<th>TASTE BUDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Te-1</td>
<td>5</td>
<td>1 day p. p.</td>
<td>10 days</td>
<td>11 days</td>
<td>2 days</td>
<td>None</td>
</tr>
<tr>
<td>Te-2</td>
<td>6</td>
<td>4 day p. p.</td>
<td>15 “</td>
<td>19 “</td>
<td>10 “</td>
<td>None</td>
</tr>
<tr>
<td>Te-3</td>
<td>4</td>
<td>16 day foetus</td>
<td>20 “</td>
<td>15 “</td>
<td>6 “</td>
<td>None</td>
</tr>
<tr>
<td>Te-4</td>
<td>5</td>
<td>18 day foetus</td>
<td>14 “</td>
<td>11 “</td>
<td>2 “</td>
<td>None</td>
</tr>
<tr>
<td>Te-5</td>
<td>8</td>
<td>6 day p. p.</td>
<td>6 “</td>
<td>12 “</td>
<td>3 “</td>
<td>None</td>
</tr>
<tr>
<td>Te-6</td>
<td>10</td>
<td>8 day p. p.</td>
<td>3 “</td>
<td>11 “</td>
<td>2 “</td>
<td>Present in all</td>
</tr>
<tr>
<td>Te-7</td>
<td>8</td>
<td>3 day p. p.</td>
<td>8 “</td>
<td>11 “</td>
<td>2 “</td>
<td>None</td>
</tr>
<tr>
<td>Te-8</td>
<td>4</td>
<td>20 day foetus</td>
<td>11 “</td>
<td>10 “</td>
<td>1 “</td>
<td>None</td>
</tr>
<tr>
<td>Te-9</td>
<td>7</td>
<td>7 day p. p.</td>
<td>5 “</td>
<td>12 “</td>
<td>3 “</td>
<td>Present in 3 cases</td>
</tr>
</tbody>
</table>

It will be observed that in all the groups of experiments the grafts were permitted to grow for a period of time in excess of that normally required for the initial appearance of taste buds. Group Te-4, for example, comprises transplants of an original foetal age of eighteen days cultivated for two weeks. Three days out of those two weeks can be thought of as belonging to the gestation period; thus the recovered graft is at a theoretical age of eleven days postpartum. It will be recalled that this age is two days beyond the average ninth-day threshold at which taste buds normally appear. Likewise the other groups of transplants, with one exception, were allowed to attain post-threshold ages of two or more days (cf. table). The exception is group Te-8 which approaches within one day of the average ninth-day threshold, but it is still within the range of observed variation in normal time of taste bud appearance.

As far as the time element alone is concerned, therefore, taste buds should have developed in all the grafts if they were capable of spontaneously doing so. On the contrary, buds appeared in only two groups of transplants; those grafted at eight (Te-6) and seven days (Te-9) of age. In the former, from two to five buds were found in all ten of the recovered grafts; buds were found in only three out of seven cases of the latter group.

This immediately suggests that determination of the taste buds, whatever the mechanism thereof, occurs between the seventh and eighth days after birth. When it is recalled that nerve fibers normally arrive at the site of future taste buds not later than the twentieth prenatal day, it appears possible that they have been involved in the determination process. However, their “function of development,” if they perform such, must not
come into play before the seventh day, otherwise taste buds should have appeared in the grafts of earlier ages.

It may still be argued, of course, that the determination mechanism is inherent within the papillary epithelium or involves some other unidentified factor, and later trophic associations with the nerve fibers are secondarily assumed. But if this were so, it is difficult to understand why gustatory organs do not appear in a graft of tissue of an early age which has been successfully cultivated beyond the bud threshold and in which the papillary epithelium has otherwise undergone an apparently normal developmental history. It may be stated in connection with this last point that a definite structural regression occurred only in the longer cultivated grafts of groups Te-2 and Te-3. The papillary epithelium in the other taste bud-free grafts appeared to have otherwise made normal developmental progress.

To recapitulate: Whereas nerve fibers arrive within a developing valvate papilla not later than the twentieth prenatal day, taste buds do not appear until some ten days later. These facts maybe interpreted as favoring the idea that nerves exert a stimulus or function of development; at least they are not contradictory, although they might be interpreted in other ways. More significant is the observation that taste buds will not reappear in a regenerating papilla unless their nervous supply is intact. But this can be considered a true function of development on the part of nerves only if it is first assumed that the regeneration of an organ is homologous to its ontogeny. The final set of experiments, therefore, lends the greatest support to the concept, for it is observed that in the nerve-free environment of the anterior eye chamber taste buds fail to appear except in those papillae transplanted just previous to the time the buds normally appear. It is thus very probably true that nerve fibers do perform a function of development upon developing gustatory organs. It is recognized, however, that the critical experiment remains to be performed: the tongue itself of the developing animal must be left intact and undisturbed while its nerve supply in some fashion is prevented from reaching it. At the present time the technical problems barring this experiment have not been solved.

*Contribution No. 286 from the Zoological Laboratories, Indiana University.
Herbst, C., Formative Reize in der Tierischen Ontogenese, Leipzig (1901).
An orientation of chromosomes that appears to be independent of meiotic synapsis in the ordinary sense is shown in a relatively large number of species. Usually this takes the form of a vis-à-vis position on the spindle and when such chromosomes are separated by a considerable distance is called “distance conjugation” (Lorbeer, '34).\(^1\) In other cases the involved chromosomes may actually come together for a period which in some instances is so brief that the movement has received the name “touch-and-go” process (Wilson, '25).\(^2\)

The mechanism involved is a very puzzling one. In a general way the explanations that have been offered fall under two headings: \((a)\) that some kind of attraction between the chromosomes is involved (Wilson, '32)\(^3\) or \((b)\) that it is mitotic forces (“centromere-spindle relationship”) that bring about this orientation and that no specific attraction is involved (Darlington, '39).\(^4\)

This latter explanation fails to account for the fact that in some instances such chromosomes approach each other before the spindle has been fully formed. Thus the \(m\) chromosomes in some cells of Alydus come to-