RENEWAL OF CELLS WITHIN TASTE BUDS

LLOYD M. BEIDLER and RONALD L. SMALLMAN

From the Department of Biological Sciences, Florida State University, Tallahassee, Florida. Mr. Smallman's present address is Pacific Science Center Foundation, Seattle, Washington

ABSTRACT

Colchicine blocks mitotic division of the epithelial cells surrounding the taste bud of the rat tongue. Response to chemical stimulation decreases 50 per cent 3 hours after colchicine injection as measured by recording the electrical activity from the taste nerve bundle. Radioautography, using tritiated thymidine, shows that those epithelial cells surrounding the taste bud divide and that some of the daughter cells enter the taste bud and slowly move toward the center. The life span of the average cell is about 250 $\pm$ 50 hours, although some cells have a much shorter and others a much longer life span. These studies suggest that the cells within the taste bud, as well as the nerves, undergo considerable change with time. Corresponding changes in function are considered.

INTRODUCTION

Organs of taste were first discovered in fish (teleosts) by Schulze (24) and were later discovered by Loven (17) and by Schwalbe (25) in mammals. In most vertebrates, the taste buds are associated with the fungiform, foliate, and circumvallate papillae on the surface of the tongue. Most taste buds consist of groups of cells collected into a bud-shaped body which opens to the exterior by a small pore (Fig. 1). Earlier researchers distinguished two types of cells in the taste bud: the taste cells, which were sensory in character and ending in so called taste hairs; and the supporting cells, which were found mainly on the periphery of the bud. Ranvier (20), von Lenhossek (28), and others observed an unusual number of leukocytes in the taste buds, indicating a degenerative process. Mitotic figures were found by Hermann (13) in the base of the bud. Other anatomists described more than two types of cells in the taste bud, and this finding together with the observation of leukocytes and mitotic figures, led Kolmer (15) to state that it was a mistake to attempt to draw distinctions between the various types of cells in the taste bud. Retzius (21) and Heidenhain (12) agreed with Kolmer and suggested that the differences in morphology of the various cells of the taste bud are due to differences in stages of growth in which they occur at a given time.

In man, and in many other animals, the taste buds on the surface of the tongue are constantly assaulted by abrasion against the teeth and hard palate; by exposure to extremes of temperature; and by flooding the tongue with various solutions. This is particularly true in man who daily abrades his tongue by talking and chewing, and subjects the taste buds frequently to draughts of hot tobacco smoke, hot coffee or tea, ice-cold alcoholic drinks, and still, after all this, retains a supply of taste buds which function.

If the nerves innervating the taste buds are damaged and degenerate, the taste buds disappear; however, if the nerve regenerates, the taste buds reappear (10, 11). It would, indeed, be unfortunate if the organs of taste were like the organs of sight or hearing which do not have the power of regeneration.

Some of the taste cells in the taste bud may be

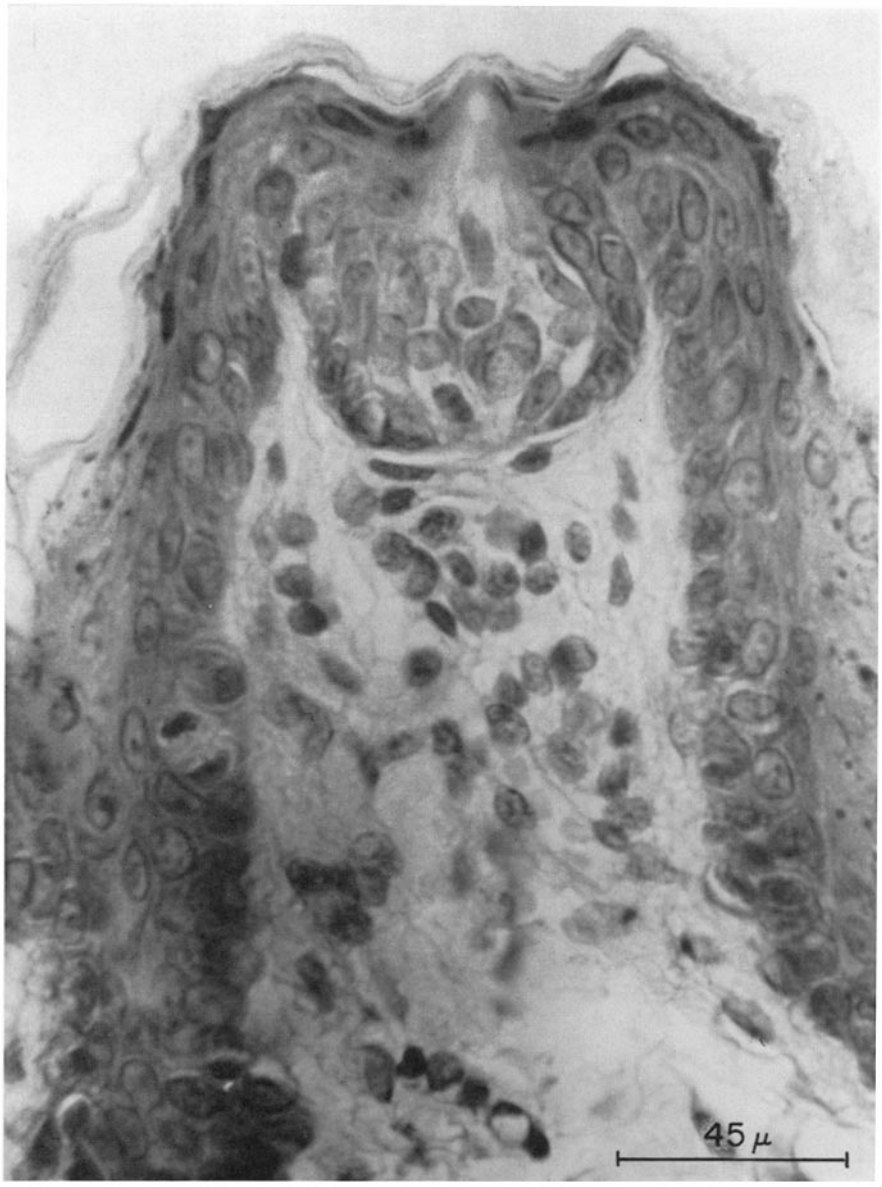

FIGURE 1 Histological section of normal taste bud of rat fungiform papilla.

injured or destroyed directly without prior damage to the taste nerve. Is it possible that the cells are continually replaced without total taste bud degeneration? If so, where do the new cells come from, at what rate are the new cells formed, and what is their life span?

To answer these questions, the functional anatomy of the taste bud was investigated using the techniques of colchicine inhibition of mitosis, electrophysiology, and radioautography.

METHODS AND RESULTS

COLCHICINE: Very small doses of colchicine, a plant alkaloid, blocks the mitotic process in cells at metaphase. Larger doses may cause damage to the general tissues; and if the dose is very large, death of the experimental animal results. In order to determine the lethal dose (LD_{50}), 50 young albino rats (250 gm) were divided evenly into five groups, the animals of four of which were injected intraperitoneally with varying amounts of colchicine: 1.0 milligram colchi-

cine per kilogram body weight, 1.25 mg/kg, 1.80 mg/kg, 5.0 mg/kg; and the fifth group served as controls. The physical activities and health of the colchicine-injected rats were compared with those of the ten control rats over a period of 10 or more days. Daily weighing of the rats injected with the lowest dose (1.0 mg/kg) showed that their weight decreased 5 to 12 per cent in comparison to the controls, with the maximum effect observed during the 4th day. The rats then quickly gained weight and by the 10th day were equal in weight to the controls. Diarrhea, accompanied by a decrease in physical activity, was often observed 24 hours after injection and continued throughout the first 4 days. The lethal dose (LD_{50}) was observed to be between 1.25 and 1.80 mg/kg (see Fig. 2) which is less than the 2.2 mg/kg lethal dose reported by Robinson and Runge (23) using an unreported injection route.

The amount of taste bud degeneration at a given colchicine dosage was determined histologically by fixing the rat tongue in formalin, staining with eosin and hematoxylin, and examining under the microscope. To determine the amount of taste bud degeneration due to 5.0 mg/kg colchicine dosage, for example, fourteen adult rats were divided into seven groups of two each as shown in Table I. The animals of six groups (the seventh was a control) were sacrificed at different times after colchicine injection and then histologically examined for normal and degenerating taste buds. All the rats that succumbed to this colchicine treatment showed numerous blocked mitotic divisions in the germinal epithelium of the fungiform

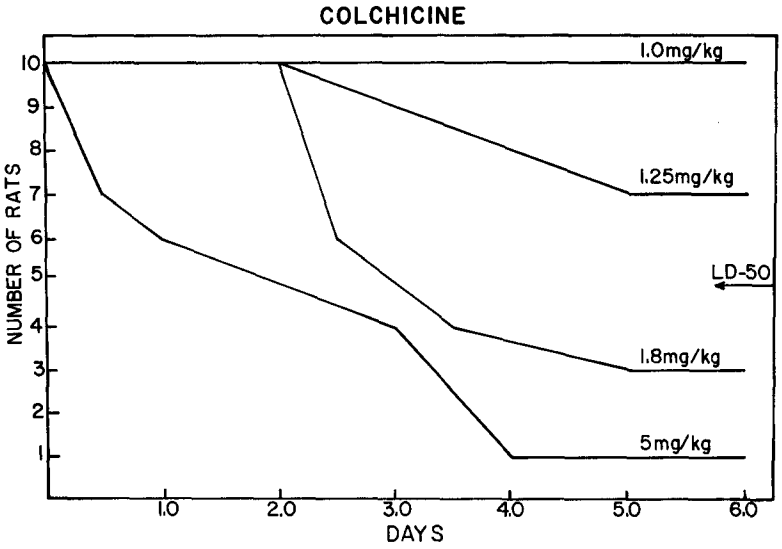

FIGURE 2 The mortality of rats after injection of various dosages of colchicine is shown as a function of days.

TABLE I

Histological Analysis of Taste Bud Degeneration at Different Times after Colchicine Injection

Time after colchicine injection (5.0 mg/kg body wt.)	No. sections counted	No. papillae found	No. normal taste buds	No. taste buds undergoing degen.	No. taste buds completely degen.	Total No. taste buds
hrs.						
Control	896	144	130	0	0	130
2	984	127	117	0	0	117
8	1000	184	1	174	4	179
10	982	132	0	133	0	133
12	984	129	34	87	0	121
24	1000	169	2	159	4	165
48	992	147	0	33	114	147

papillae as well as around the taste bud. Histological evidence for such degeneration was seen 8 hours after injection, although at that time only a few taste buds were completely degenerated. Only after 48 hours were most of the taste buds degenerated. The average number of fungiform papillae found in these fourteen rats was 149 which is comparable to the value of 179 found by Fish, Malone, and Richter (9). 144 of the 149 papillae contained a single taste bud; double taste buds were rarely seen. By repeating the above experiments with different dosages, it was found that 1.0 mg/kg colchicine was the minimum dose to completely block mitotic division in the fungiform papillae, which agrees with similar values published by Bertalanffy and Leblond (5).

Histologic examination of the tongue, after intraperitoneal injection of colchicine (1.0 mg/kg), showed an accumulation of mitotic figures around the taste bud, increasing in number until 6 hours after injection, then decreasing in number to become normal 12 hours after injection. Because colchicine, at this dose level, does not attain its full effectiveness until 1 hour after injection and begins to lose effect after 6 hours, the rate of mitotic figure accumulations was tabulated between the 2nd and 5th hour after injection, as shown in Table II. To find the rate on an hourly basis, the difference between the means of the 2- and 5-hour periods was divided by three. Thus, 1.16 cells per hour entered mitosis in the estimated 34 germinal cells surrounding the taste bud, or 2.9 per cent of these cells entered mitosis each hour. This value is in contrast to 4.56 per cent of the cells of the germinal layer of the general epithelium of the superior surface of the tongue that enters mitosis each hour. No mitotic figures were ever seen inside the taste bud. In those cases in which such figures were thought to be inside the bud on first examination, more careful analysis showed that such sections were tangential to the bud and included a number of germinal cells surrounding the taste bud, and that it was these germinal cells which were undergoing mitotic division and not cells within the taste bud. This is a possible explanation for the dividing cells seen at the base of the taste bud by Hermann (13) and in the center of the bud by Beidler *et al.* (1).

The influence of extraperitoneally injected colchicine upon the neural activity of the taste cells was determined electrophysiologically. Young adult albino rats (250 gm) were injected with 5.0 mg/kg colchicine in order to see the maximum effect of colchicine at doses above the LD_{50}. At two to 20 hours after injection the animals were anesthetized with Nembutal or urethane. The chorda tympani was exposed in the lower jaw where the nerve passes from the bulla of the ear to the tongue, and the neural responses to stimulation of the taste buds of the fungiform papillae were recorded. The relative change in magnitude of response to 0.1 M NaCl stimulation of the tongue after colchicine injection is shown in Table III. In many such preparations the response to sucrose stimulation increased 10 to 20 per cent in magnitude above that of control animals, and a fair amount of spontaneous neural activity was observed

TABLE II

The Rates of Accumulation of Mitotic Figures after Colchicine Injection

Taste bud germinal layer		Germinal layer of the general epithelium on the superior surface of the tongue	
No. of mitotic figures	Frequency of occurrence	Cells in mitosis	Frequency of occurrence
Rate at 2 hours after injection (1.0 mg/kg body wt.)			
		Per cent	
0	11	0	16
		3	3
1	57	4	29
		5	36
2	30	6	20
		7	15
3	4	8	18
		9	12
		10	13
		11	8
$\bar{X}$ = 1.26 or 3.1 per cent		12	9
S.D. = 0.7		13	4
		14	2
		15	3
		$\bar{X}$ = 6.8 per cent	
		S.D. = 1.54	
Rate at 5 hours after injection			
2	4	11	2
		14	2
3	15	15	2
		16	3
4	26	17	6
		18	3
5	31	19	3
		20	3
6	10	21	6
		22	1
7	11	23	2
		24	1
8	4	25	5
		30	1
		33	1
$\bar{X}$ = 4.76 or 11.9 per cent			
S.D. = 2.18		$\bar{X}$ = 20.5 per cent	
		S.D. = 4.44	

while the preparation was declining in sensitivity and disappeared 8 hours after colchicine injection. It was possible to obtain a response to tactile stimulation of the surface of the tongue by brushing with a probe, even after all taste responses were abolished.

Similar results were obtained with doses of colchicine well below the LD_{50}; however, no such effects were observed when the dose was decreased below 1.0 mg/kg. The tongues of all rats were fixed in formalin, stained with eosin and hematoxylin, and examined histologically. In those cases where taste responses were completely abolished, signs of degeneration appeared in the taste buds 8 hours after colchicine injection. The amount of degeneration increased with time, and taste buds ⅔ empty to completely evacuated were seen after 24 to 48 hours. The bud shape of the sense organ was still maintained with the same dimensions as the controls, indicating that the cells of the taste bud are under no mechanical pressure from the surrounding cells, the so called germinal cells.

TABLE III

Relative Change in Magnitude of Neural Response to Stimulation of Tongue after Colchicine Injection

Time after injection (5.0 mg/kg body wt.)	Electrophysiological response to 0.1 M NaCl
hrs.	*per cent*
0	100
3	50
6	10
8	0–10
10	0

No change in magnitude of electrophysiological responses to chemical stimulation, in comparison to control animals, was observed after saturated solutions of colchicine were flowed over the surface of the tongue for up to 3 hours' duration. This is probably due to the inability of colchicine to enter the taste bud. Six rats were anesthetized and normal taste responses were recorded from the chorda tympani. Then

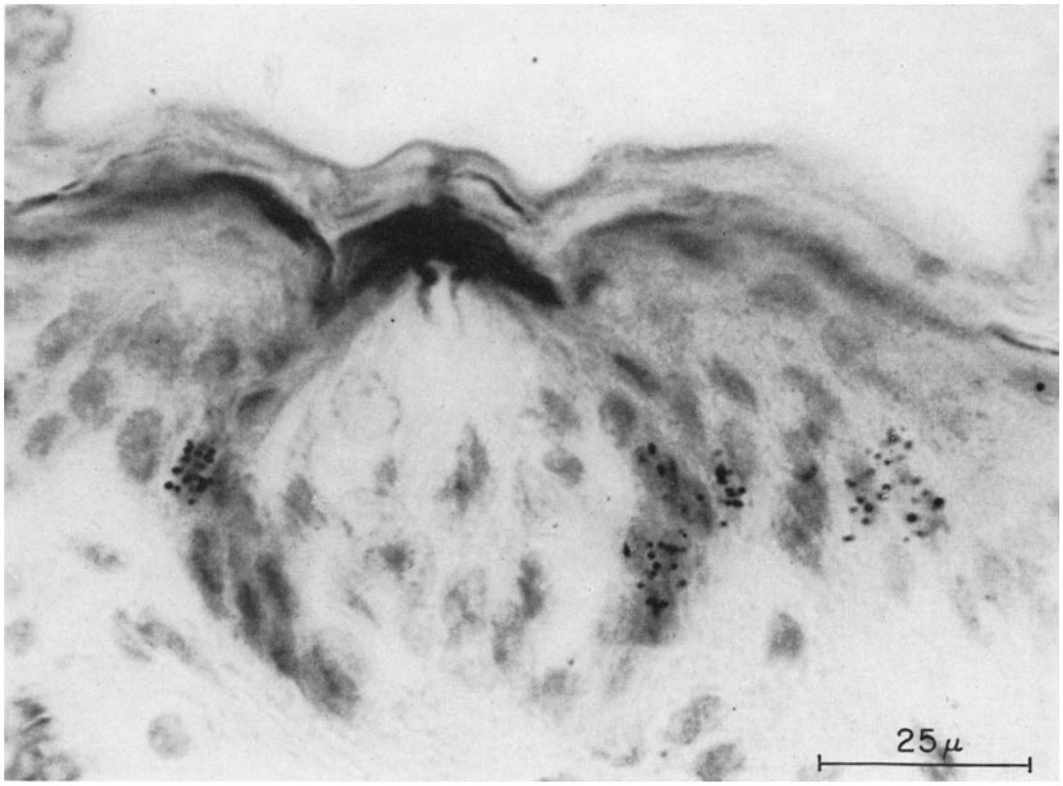

FIGURE 3 Radioautogram of taste bud of rat fungiform papilla, showing labeled nuclei 24 hours after tritiated thymidine injection. Note labeled cells immediately surrounding taste bud as well as those along papilla wall.

5.0 mg/kg colchicine was injected and the recording continued up to 8 hours. It is of interest that in none of these experiments did it appear that colchicine had an effect on the response after the animal was anesthetized and the chorda tympani cut.

TRITIATED THYMIDINE: To determine whether cells migrate into the taste bud, and, if so, at what rate and from what source, rats were injected intraperitoneally with 0.7 μc of tritiated thymidine at 1.9 c/mM specific activity. Thymidine is incorporated into the animal's "thymidine pool" and picked up by cells during their DNA synthesizing period prior to mitotic division (see Leblond *et al.*, 16). The tongues of these animals were removed at periods of ½, 1, 2, 5, 24, 50, 100, 200, 300, and 600 hours after injection, fixed in formalin, embedded in paraffin, sectioned, and the sections affixed to glass slides. The sections on slides were then deparaffinized and coated with Kodak type NTB emulsion to make radioautograms. The radioautograms were developed after about 14 days' exposure, stained with haematoxylin and eosin, covered, and examined microscopically.

The radioautographic data used in this report were compiled after detailed examination of the tongues of 58 rats cut into 10-micron sections, which resulted in a total of over 9,600 sections. In all cases in which the number of mitotic figures per taste bud or the number of labeled cells per bud was counted, any bud which did not show clearly on each section through its diameter was discarded from the tabulation.

A few labeled cells are seen within ½ hour after injection of tritiated thymidine, but a large number of labeled cells are seen 1 hour later in the germinal layer surrounding the taste bud as well as in other areas of the papillae. Most of the dividing cells surrounding the taste bud do not send their labeled daughter cells into the taste bud. The germinal cells that surround the taste bud overlap as do shingles on a roof, as seen in Fig. 3. In 24 hours (Fig. 3) most of the labeled cells still surround the taste bud although a few may be seen to have entered it. Only those cells immediately adjacent to the taste bud actually enter the bud, while those in other layers move outward and upward to form the epithelial layer of the upper end of the papilla.

At 50, 100, and 200 hours, after injection of isotope, the radioautograms indicate a general migration of the labeled cells toward the center of the taste bud, as shown in Fig. 4, although many cells never reach the center before they degenerate. At 400 hours after

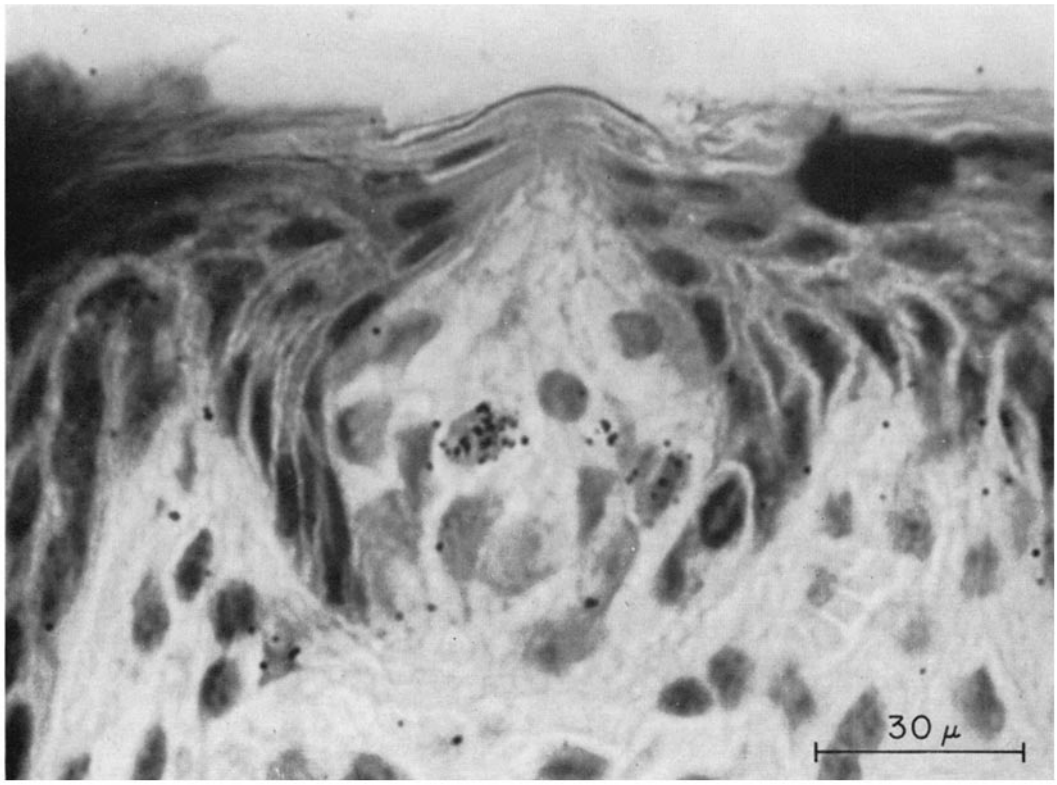

FIGURE 4 Radioautogram of taste bud of rat fungiform papilla, showing labeled nuclei 100 hours after tritiated thymidine injection.

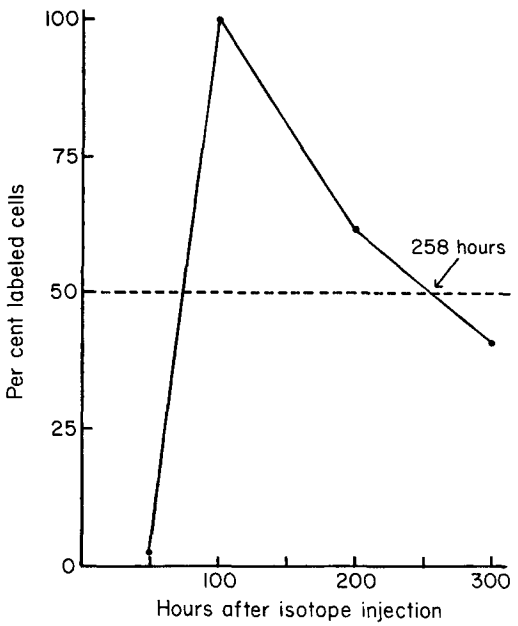

FIGURE 5 Graph showing per cent labeled cells (number of labeled cells found per number of taste buds examined) as a function of time after injection of tritiated thymidine. The values were normalized so that the above ratio at 100 hours was equated to 100 per cent.

injection the tritiated thymidine in most, but not all, of the taste buds appears as spots scattered throughout the taste bud but not associated with any cell nucleus. Several such studies of entrance and disappearance of labeled cells within the taste buds were undertaken and all indicated that the average life span of a taste bud cell is about 250 ± 50 hours. The data obtained from the examination of 253 taste buds in one series of experiments are shown in Fig. 5. Half the labeled cells were present in the taste buds about 258 hours after isotope injection. In some cases, an accumulation of tritiated thymidine is seen in the taste pore. DeLorenzo (6–8) also saw evidence of cell degeneration within the taste bud. The rate of entry of the labeled cell into the taste bud is about one every 10 hours.

DISCUSSION

How can we explain the rapid decrease in neural response recorded after injection of 5 mg/kg colchicine? We know that the existence of the taste bud depends upon the presence of the chorda tympani nerve endings. We also know that new cells move into the taste bud at a rate of one every 10 hours, and, to maintain the cells of the taste bud in a steady state, cells must degenerate at the same rate.

The germinal layer making up the wall of the taste bud is rigid enough to maintain the rounded shape even when the bud itself is completely empty of cells. Thus, it is quite conceivable that as soon as one taste cell degenerates and a new cell is prevented by colchicine from entering the bud, the space between the remaining cells increases. This occurs within 10 hours after colchicine injection. DeLorenzo (6), however, has shown that the taste cells are normally in close proximity to one another so that one synaptic nerve ending may contact the surfaces of up to three adjacent cells. If the distance between the taste cells increases by a few hundred Angstroms, then contact between nerve ending and taste cell diminishes. This may lead to an abrupt decrease in neural response to taste bud stimulation as well as a slow increase in rate of taste cell degeneration. Another possible cause for taste cell inactivation is the toxicity of colchicine at high (5.0 mg/kg) dose levels due to effects other than mitotic blocking. This is particularly pertinent since the inactivation is not readily noticed at dose rates of 1.0 mg/kg, although histologic examination indicates some mitotic blocking at metaphase. The blocking of mitosis by 1.0 mg/kg colchicine begins to decrease after 6 hours, so that a turnover rate of one new cell per 10 hours is not seriously affected. It is relevant that Singer *et al.* (26) showed that peripheral nerve destruction occurred after the normal forelimb of the newt was infused with a dilute colchicine solution. Inhibition of forelimb regeneration by colchicine was thought to be due primarily to nerve destruction and secondarily to mitotic inhibition.

The nuclei of those cells that were synthesizing DNA at the time tritiated thymidine was administered became tagged. This occurred within the 1st hour after injection. Since few labeled cells are observed within the taste bud after the first 24 hours, the release of cells from the area of the germinal epithelium surrounding the taste bud to the taste bud itself depends upon the labeled cell having gone through mitosis and some later transition phase. After the labeled cell enters the taste bud, it presumably is innervated by local nerve endings and differentiates into a taste cell with microvilli. A summary of these phases is shown in Fig. 6. Thus, the cell matures,

plays a role in taste physiology, and finally decays, all within an average of approximately 250 hours. In the meantime, the population of mother cells is undergoing mitotic division at an average rate of 1.16 cells per hour, but only about one of the cells enters the taste bud every 10 hours. The average life span of a taste cell is longer than that found for mouse intestinal epithelial cells (60 hours) by Quastler and Sherman (19). DeLorenzo (8) "repeated Beidler's rat experiments in the rabbit" and concluded that the experiments "do suggest a rather rapid turnover in the foliate papillae." His preliminary data suggested that the number of labeled cells within the taste bud of the rabbit foliate papilla is greatest between 100 and 200 hours after tritiated thymidine injection, which is slightly lower than previous reports (2, 3) on the cells of the taste bud of the rat fungiform papilla would indicate. All values for the taste bud cells are averages for normal rat tongues. How external or internal influences may change the taste bud cell life span or the mitotic rates was not studied.

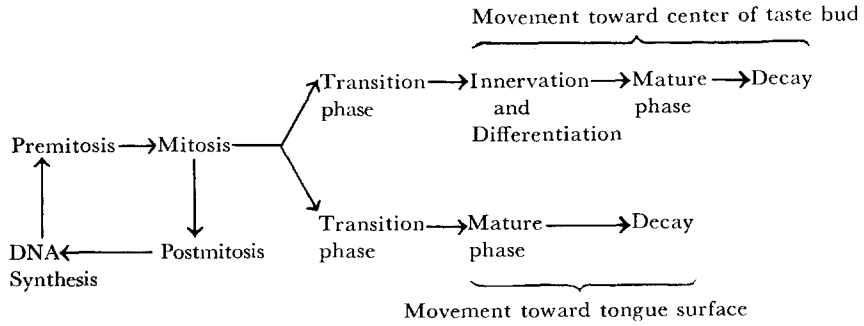

FIGURE 6 Flow diagram of cell proliferation and renewal associated with taste buds of rat.

Since only the layer of normally dividing cells was used in the determination of mitotic counts, the turnover time for the total superior surface of the tongue cannot be accurately calculated. Thus, 4.56 per cent of the cells of the germinal layer of the superior tongue surface of the rat was found to be passing through mitotic division each hour, although Bertalanffy (4) stated that the turnover time for the total cell population of the superior tongue surface of the mouse was between 4 and 8.4 days.

Electron microscopists often describe two types of cells in the taste bud: one with a light, vesicular cytoplasm and the other with a dense cytoplasm and an elongated, irregularly shaped nucleus. According to Rhodin (22), both cells have microvilli that project into the taste pore and "in view of the fine structural findings, it seems safe to assume that both types of cells are taste receptors." Kimura and Beidler (14) as well as Tateda and Beidler (27) have recorded changes in the resting potential of cells in the taste bud in response to a number of taste stimuli. Whether fine nerve fibers that penetrate into the taste bud but do not synapse with taste cells also respond to taste stimuli is not known. From the above considerations, one may assume that the cells which are renewed continuously are indeed sensory taste cells. If taste cells are renewed, age, and then die, what happens to their taste sensitivities during this time and how does the innervating nerve keep informed of any changes in sensitivity? The answers are not yet known, but it is quite possible that the individual taste cell does change its sensitivity to various taste qualities as the cell ages. For example, young cells might be more sensitive to sugars than older cells that are more sensitive to bitters. Such changes could result from molecular changes in cell membrane structure. When a new cell enters the taste bud, it is likely that the closest nerve fiber sends out an axon extension and makes contact with the cell. As the cell ages and moves away from the taste bud periphery, other axons closer to the center of the bud may innervate in turn. Thus, one nerve fiber may always innervate new cells, and another, old cells. In this way, the individual taste cells of the total population would be in continuous flux, but the population as a whole would maintain static taste characteristics. Indeed, electrophysiological recording from a single chorda tympani nerve fiber would not be expected to present any evidence for the dynamic properties of the taste cells. It is known, however, that many free nerve endings show continual motion and

that regenerating rat nerves grow at a rate of 137 μ/hour or 2.3 μ/minute (18), which is fast compared to that which would be necessary to innervate new cells entering the taste bud.

The shape of the curve in Fig. 5 which relates the number of labeled cells in the taste bud to time is not what is expected if the cells all have approximately the same life span. In this case, the per cent labeled cells within the taste bud should remain constant for a number of hours and then decline with the same slope as that with which the labeled cells entered the taste bud originally. The solid curve of Fig. 7 indicates such a theoretical relation in which half the labeled cells was assumed to disappear in 250 hours. The particular shape of this curve is due to the fact that the taste bud volume is rather constant and that if a new cell enters the taste bud another must leave at the same time. If, however, the cells of the taste bud are continually damaged by exposure to harsh stimuli, and if this damage to the cells occurs randomly, then once a labeled cell enters the taste bud it is exposed to the same hazards as all of the other cells of the taste bud. One would then expect the number of labeled cells that disappear at any time, $\frac{dL}{dt}$, to be related to the total number of labeled cells available, (L), within the taste bud, or $\frac{dL}{dt} = -AL$, where A is a constant of proportionality whose magnitude is dependent upon the amount of hazard the cells are exposed to. Mathematical integration of the above results in a logarithmic relation between the number of labeled cells within the taste bud and the time after their entrance, or $L = L_0 e^{-AT}$. The dashed curve of Fig. 7 indicates such a theoretical relation in which half the labeled cells was assumed to disappear in 250 hours and in which the normal life span of a labeled cell without exposure to injury was assumed to be about 300 hours. Note that the exponential decline of this curve is similar to that of Fig. 5 which is based upon experimental data. This similarity agrees with the concept that the cells within the taste bud do not all have the same life span but that the cells may be injured at various times so that some may live only a few hours and others many hundreds of hours, although the average life is about 250 hours.

The data in this paper suggest that researchers working with taste buds are dealing with a dynamic rather than a static system. Both the taste bud cells and the nerve endings within the bud move rather fast. In addition, the microvilli of the taste cells project into the mucus covering the tongue surface. Whether these microvilli can be repaired within the life span of the cell is not known.

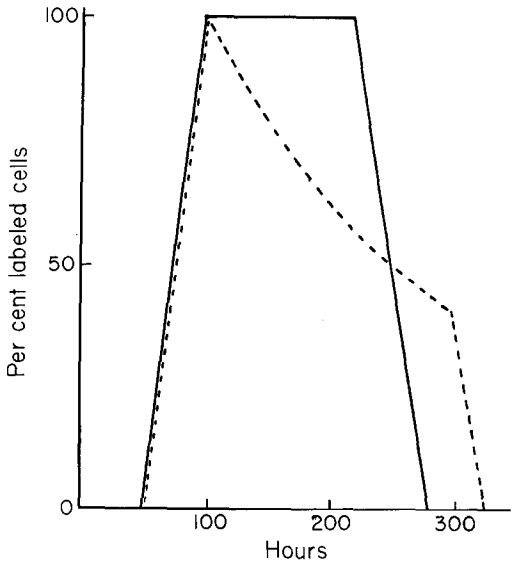

FIGURE 7 Graph showing per cent labeled cells in taste bud as a function of time. Solid curve assumed all cells have same life span of 250 hours. Broken curve assumed all cells would have normal life span of 300 hours but are destroyed randomly by harsh stimuli so that only half remain after 250 hours.

Important questions yet to be answered are what regulates the production of new cells and how are the cells differentiated or modulated to form taste cells? What is the role of the innervating nerve fibers?

This research was supported in part by the Office of Naval Research under Contract Nonr 589(00) and by the National Science Foundation Grant No. G-14334.

The authors wish to acknowledge the skilled technical assistance of Mr. Roderick Muntz. They have also received generous help from Dr. M. Nejad and Dr. H. Tateda.

Preliminary reports of this work appeared in *Fed. Proc.*, 1960, **19**, 302, by Beidler, L. M., Nejad, M., Smallman, R., and Tateda, H.; Taste receptor stimulation, *in* Progress in Biophysics and Biophysical Chemistry, London, Pergamon Press, 1961, by Beidler, L. M.; and Biophysical approaches to taste, *Am. Scientist*, 1961, **49**, 421, by Beidler, L. M.

Received for publication, March 22, 1965.

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