

KEY WORDS: tongue; heterogeneity; differon.

Taste buds of the mammalian tongue consist of cells that differ in the staining properties of their cytoplasm and in their ultrastructural features [4, 5, 8, 10, 12-14]. Two hypotheses have been put forward to explain the heterogeneity of the cell composition of taste buds. According to one of them, dark, pale, and intermediate cells are different stages of differentiation of the same cell type (differon). It is suggested that intermediate cells are formed from dark cells, and later these differentiate into pale cells, which are the terminal stage of development of the taste bud cells [8, 11]. According to the other hypothesis, dark, pale, and intermediate cells are independent cell types [7, 9]. In the present experiments, in which the trophic influence of nerves on the taste buds was blocked, changes in the state of the taste receptors of the foliate papillae of the rat tongue were demonstrated [1]. Consequently, and taking account of heterogeneity of the taste bud cell population, it was decided important and interesting to study whether the neurotrophic influence is exerted differentially on dark, pale, and intermediate cells.

The aim of this investigation was to study the ratio between numbers of different cells in taste buds of the foliate papillae of the rat tongue during blocking of the trophic influence of nerves by colchicine. The data obtained on changes in the relative proportions of taste bud cells were used to analyze existing views on the connection between dark, pale, and intermediate cells in the mammalian taste receptor.

#### EXPERIMENTAL METHOD

Under urethane anesthesia (50  $\mu\text{g/kg}$ ) a 5 mM solution of colchicine (from Merck, West Germany) was applied for 10 min to the glossopharyngeal nerve of albino rats weighing 150-200 g in the submandibular region. The posterior region of the foliate papillae of the tongue on the ipsilateral side was isolated 5, 7, and 10 days after the operation. The posterior region of papillae on the opposite side and of intact animals served as the control. Material was fixed in 2.5% glutaraldehyde and subsequently treated in 1%  $\text{OsO}_4$  solution at 4°C and pH 7.4. In semithin sections stained with methylene blue the area of the buds, the number of cells in a section through one bud, and the number of dark, pale, and intermediate cells were counted. Only oblique sections through the taste buds were used for this purpose.

#### EXPERIMENTAL RESULTS

Application of colchicine to the nerve caused a decrease in the area of cross section of the taste buds, which was observed on the 10th day after the operation. At this time, the area of the taste buds on the ipsilateral side was 36.1% less ( $P < 0.001$ ) than on the contralateral side. Comparison of the number of cells per section through one bud on the ipsilateral and contralateral sides 5 and 7 days after application of colchicine to the nerve revealed no significant differences. Ten days after the operation the total number of cells in the taste buds was reduced by 20.0% ( $P < 0.001$ ) compared with the number on the contralateral side. Taste bud cells of foliate papillae of the rat tongue differed in the density of their cytoplasm (Fig. 1). Counting cells in intact animals showed that there were 61.8% of dark, 25.5% of pale, and 12.5% of intermediate cells [1]. Application of colchicine to the glossopharyngeal nerve led to a fall in the relative number of dark cells and a rise in the number of intermediate cells (Fig. 2). Similar changes in the numbers of dark and intermediate cells

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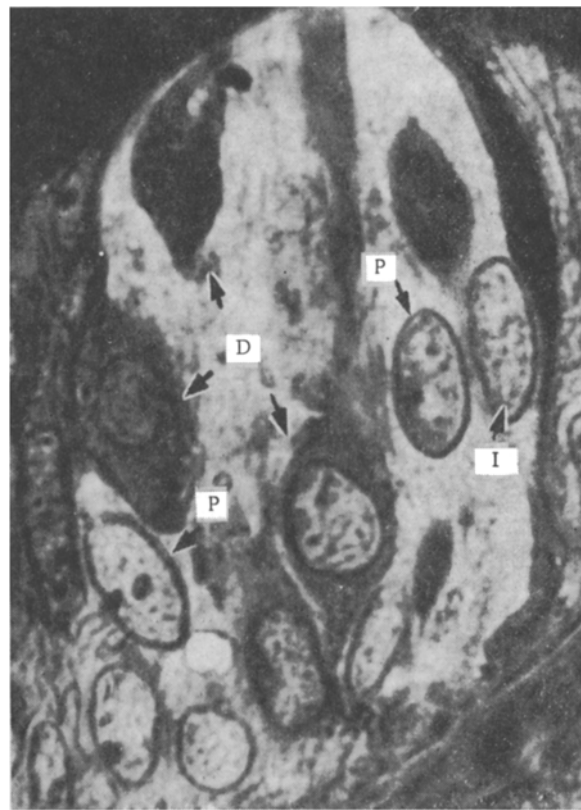


Fig. 1. Taste bud of foliate papilla of tongue of intact rat. D) Dark cell, I) intermediate cell, P) pale cell. Methylene blue, 342 $\times$ .

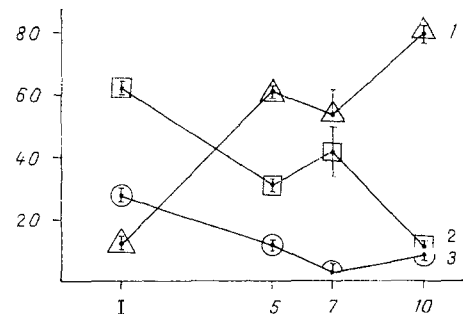


Fig. 2. Effect of colchicine application to nerve on relative numbers of intermediate (1), dark (2), and pale (3) cells in taste buds of foliate papillae of rat tongue. Abscissa, time after colchicine application (in days), ordinate, relative number of cells (in %). 1) Intact animals.

were observed after division of the glossopharyngeal nerve [1], while an increase in the relative number of intermediate cells is proportional to the decrease in the number of dark cells, as shown in the taste buds of the vallate papilla of the rat tongue after removal of the salivary glands [3]. Allowing for changes in area of the taste buds and in the total number of cells in the bud, the absolute number of dark and intermediate cells was counted in the epithelium of the foliate papillae at different times after application of colchicine to the nerve. The results of these counts showed a decrease in the number of dark cells and an increase in the absolute number of intermediate cells by the 10th day after the operation (Fig. 3).

Let us see how these facts can be correlated with the two hypotheses on different taste bud cells. The first hypothesis assumes that all cells of taste buds constitute one differon [8, 11]. If the neurotrophic control factor acts on this differon at only one point, permit-

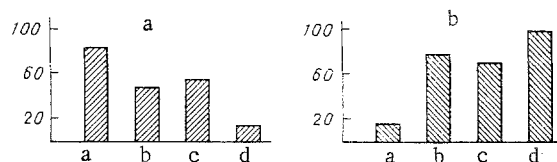


Fig. 3. Effect of colchicine application to nerve on absolute number of dark (a) and intermediate (b) taste bud cells. Abscissa: a) intact animals, b) 5 days, c) 7 days, d) 10 days after colchicine application; ordinate, absolute number of cells per  $10^5 \mu^2$  area of taste buds.

ting differentiation of dark cells from undifferentiated precursor cells, our results showing an increase in the number of intermediate cells and a simultaneous decrease in the number of dark cells, contradict this hypothesis: Different taste bud cells represent different stages of differentiation of the same cell type. The decrease in the number of dark cells under these circumstances ought to lead to a decrease in the number of intermediate cells, but this was not observed in the present experiments. Meanwhile, the different direction of the changes in number of dark and intermediate cells does not contradict the other hypothesis of independent differentiation of different cell types in the taste bud from the stem cell [7, 9, 10]. However, the results cannot be interpreted unequivocally within the framework of this hypothesis. It may be that application of colchicine to the nerve depresses axonal transport of various chemical factors. One of these is permissive for differentiation of dark cells, whereas the other inhibits differentiation of intermediate cells. A different explanation but within the framework of this same hypothesis of independent differentiation, postulates that when the trophic influence of the nerves is blocked differentiation of dark cells is inhibited, and with the participation of chemical factors produced in them, similar to chalcones, they inhibit proliferation and differentiation of the intermediate cells. The absence of significant changes in the number of pale cells after colchicine application to the nerve may be associated with their longer life span [6] than that of other taste bud cells. Consequently, the results now obtained do not support the hypothesis that morphologically different taste bud cells belong to the same cell type.

#### LITERATURE CITED

1. Yu. A. Chelyshev, T. L. Zefirov, and Z. Kh. Timergaleeva, *Byull. Eksp. Biol. Med.*, No. 3, 92 (1982).
2. M. L. R. Angulo, B. F. Sanchez, and E. L. Rodriguez-Echandia, *Cell Tissue Res.*, 192, 67 (1978).
3. J. Cano, C. Roza, and E. L. Rodriguez-Echandia, *Experientia*, 34, 1290 (1978).
4. A. J. De Lorenzo, *J. Biophys. Biochem. Cytol.*, 4, 143 (1958).
5. A. I. Farbman, *Dev. Biol.*, 11, 110 (1965).
6. A. I. Farbman, *Cell Tissue Kinet.*, 13, 349 (1980).
7. S. Fujimoto and R. G. Murray, *Anat. Rec.*, 168, 393 (1970).
8. T. Iwayama, *Anat. Rec.*, 130, 283 (1958).
9. R. G. Murray, in: *Handbook of Sensory Physiology*, Berlin (1971), pp. 31-50.
10. R. G. Murray, A. Murray, and S. Fujimoto, *J. Ultrastruct. Res.*, 27, 444 (1969).
11. M. L. Rodrigo-Angulo and E. L. Rodriguez-Echandia, *Trab. Inst. Cajal Invest. Biol.*, 69, 163 (1977).
12. H. A. Scalzi, *Z. Zellforsch.*, 80, 413 (1967).
13. M. Takeda, *Acta Anat. Nippon*, 47, 325 (1972).
14. M. Takeda and T. Hoshino, *Arch. Histol. Jpn.*, 37, 395 (1975).