

Fig. 1. Absorption spectrum of chlorophyll a in diethyl ether.

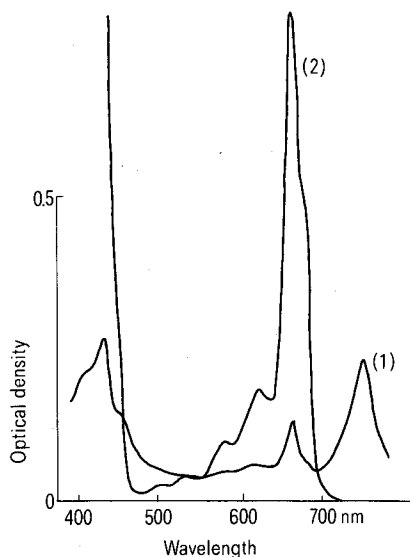


Fig. 2. (1) Absorption spectrum of suspension/solution of chlorophyll a in isooctane; (2) Same sample heated 10 min to 80°C.

nol: petroleum ether 4:1. After removal of cell debris by centrifugation, the mixture was diluted with saturated NaCl solution until most of the pigments were in the petroleum ether layer. This was separated from the water, centrifuged, chromatographed on commercial powdered sugar, and eluted with petroleum ether plus 0.5% n-propanol. The fraction containing chl a was extracted successively with 50, 60, 70 and 80% methanol, and washed several times with water. When the solution was kept at  $-20^{\circ}\text{C}$  overnight, m-chl a precipitated. Small amounts of carotenoids did not interfere.

Figure 1 shows the spectrum of m-chl a, redissolved in diethyl ether. It is identical with the standard spectrum<sup>5</sup>. The absorption spectrum of the suspension of m-chl a in isooctane is shown in figure 2. The peak at 745–746 nm proves the presence of m-chl a. Short heating of the suspension to  $80^{\circ}\text{C}$  converts this m-chl a to the monomer with its well-known peak at 662 nm. Microcrystallinity was also confirmed with X-rays; the  $\text{CrK}_{\alpha}$  powder pattern agreed with that obtained by Donnay<sup>6</sup>. Elemental analysis and IR spectrography proved that the chl a was intact, i.e. that the phytol chain had not been split off.

The preparation of high-purity microcrystalline chl a in mg amounts from *Anacystis nidulans* by this method requires less than 1 day. Moreover, extraction does not involve breaking of chloroplasts, with consequent danger of alteration of the chlorophyll, owing to contact with substances contained in the cytoplasm.

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## Effects of selective removal of the salivary glands on taste bud cells in the vallate papilla of the rat<sup>1</sup>

J. Cano, C. Roza and E.L. Rodríguez-Echandiá

Departamento de Morfología, Facultad de Medicina, Universidad Autónoma de Madrid, Madrid (España), 8 March 1978

**Summary.** The in-block removal of the main salivary glands produced a significant increase in the rate of development of the intermediate type of taste bud cells. Such effect was reproduced by removal of submaxillary-sublingual glands. Removal of parotid glands was not effective.

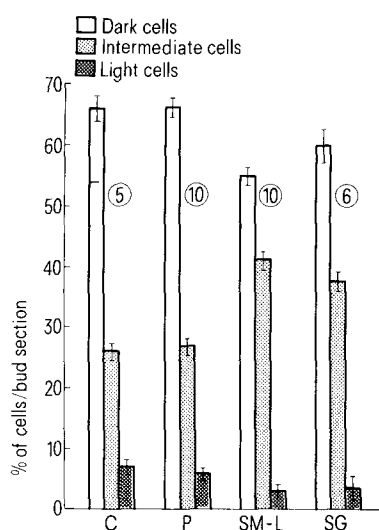
Taste buds in mammals contain distinctive cell types<sup>2,3</sup>. These are referred to as dark cells (type I), light cells (type II) and intermediate cells (type III). In this particular system there is a continuous renewal of cells. Dark cells differentiate from basal cells (type IV) or capsular cells<sup>4,5</sup> and subsequently mature into intermediate cells<sup>6</sup>. Light cells are aged intermediate cells close to leaving the system by desquamation<sup>6</sup>. All cell types in taste buds represent different stages of a single cell strength.

Inasmuch as the rates of development of dark, light and intermediate cells are believed to remain constant<sup>7,8</sup>, it was assumed that any experimental alteration of their life cycle could be properly shown by bud cell counts. Of course, favourable technical conditions are essential to achieve comprehensive results.

The effect of the removal of the main salivary glands on the differentiation and maturation of taste bud cells was described elsewhere<sup>8</sup>. The present report is an attempt to localize the secretion site of the gland factors responsible for such effects through selective removal of parotid, submaxillary and sublingual glands.

31 Wistar rats, weighing 200–300 g, were used. These were anesthetized with ether and the salivary glands were surgically exposed. A bilateral removal of parotid, submaxillary and sublingual glands was performed in 6 animals. In a 2nd group of animals, the parotid glands (10 rats) or the submaxillary-sublingual glands (10 rats) were selectively removed. Animals of a 3rd group (5 rats) were sham operated (control group). All rats were sacrificed on the 15th day after the operation; the vallate papilla was

removed and fixed in the Karnowsky mixture<sup>9</sup> buffered with 0.2 M collidine. The material was postfixed in OsO<sub>4</sub>, dehydrated and embedded in Epon 812. Serial sections, each 1 µm in thickness, were obtained and stained with toluidine blue-borax (pH 11). Only obliquously sectioned buds were selected for counting of cells. The longitudinal and cross-sectioned buds were not suitable for this purpose. The in-block removal of parotids, submaxillary and sublingual glands significantly increased the rate of intermediate cells with respect to control preparations (figure). No changes in the rates of dark and light cells were detected. Bilateral removal of parotid glands did not produce structural changes in the taste buds (figure). Bilateral removal of submaxillary and sublingual glands dealt with an increase in the rate of intermediate cells comparable to that observed after the in-block removal of the salivary glands (figure). Although dark cells did not vary in number with respect to the control, their percentage decreased proportionally to the increase in intermediate cells. Present results support previous investigations on the effect of sialectomy on differentiation and maturation of taste bud cells in the vallate papilla of the rat<sup>8</sup>. The significant increase of intermediate cell rates in the 15-day sialectomized rats points to a stimulation of bud cell maturation.



Effects of the salivary glands on taste buds cells percentages in the vallate papilla of rat. C control buds; P, animal deprived of parotid glands; SM-L: submaxillary sublingual glands removal; SG: in-block removal of the main salivary glands; Number: number of animal. Mean±SEM.

Taste bud cells have a mean life of approximately 10 days<sup>4</sup>, so the absence of a comparable decrease in the number of dark cells was regarded as due to a simultaneous stimulation of cell differentiation. The absence of significant changes in the rate of light cells suggests that the involution of the taste bud cells was not primarily affected by sialectomy. This, however, does not preclude the possibility that changes in this cell stage might be found in longer-term experiments.

The bilateral removal of parotid glands did not affect the life cycle of taste bud cells, but removal of submaxillary and sublingual glands produced changes comparable to those observed after sialectomy. This finding suggests that these salivary glands are involved in the mechanism controlling the life cycle of taste bud cells. Whether the products responsible for such an effect are secreted, concentrated or activated by submaxillary and sublingual glands, cannot be ascertained at present. It has been shown that all of these processes take place in the salivary glands. The submaxillary glands have 'hormonal functions' through secretion of the nerve growth factor<sup>10</sup>, the epithelial growth factor<sup>11</sup>, steroids<sup>12</sup> and other products. On the other hand, the submaxillary gland is known to concentrate some circulating hormones<sup>13</sup>. Finally, it has been reported that testosterone<sup>14</sup> and progesterone<sup>15</sup> are transformed into other products in the submaxillary glands. Additional investigation is needed to gain a better understanding of the mechanism by which salivary glands contribute to the regulation of the life cycle of taste bud cells.

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## Directed biosynthesis of analogues of ergot peptide alkaloids with *Claviceps purpurea*

E. Beacco, M.L. Bianchi, A. Minghetti and C. Spalla

*Farmitalia, Viale E. Bezzi 24, I-20146 Milano (Italy), 17 March 1978*

**Summary.** Analogues of peptide ergot alkaloids can be obtained by feeding a producing culture of *Claviceps purpurea* with an analogue of one of the amino acids of the peptide chain of the alkaloid.

It is known that the biosynthesis of the peptide antibiotics is catalyzed by polenzyme complexes, and that these enzymes have a rather broad specificity with the consequence that structurally similar amino acids can replace certain of

the amino acid residues of the peptide chain during the antibiotic biosynthesis<sup>1</sup>. The ergot alkaloids of the ergotamine and of the ergotoline groups are formed by a moiety of lysergic acid linked to a