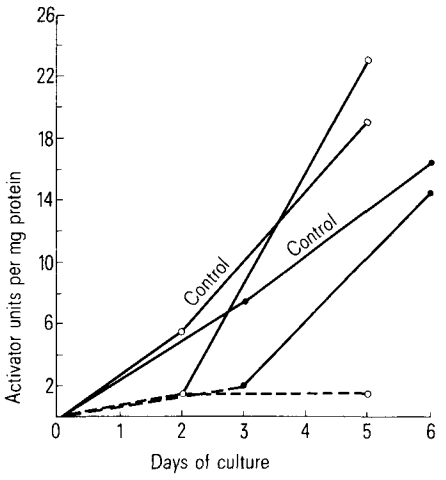
culture period at which the assay was performed. At lower cycloheximide concentrations results were less consistent. 2 further experiments are presented in the figure. They show that the same pattern was obtained when the results were calculated on the basis of amount of protein present in explants instead of their wet wt. Furthermore, in these experiments, cycloheximide containing medium was removed from some explants after 2, respectively 3 days of culture. Explants were washed 3 times with Hank's BSS and then were re-supplied with fresh normal medium. These cultures regained their capacity to produce TPA, while parallel cultures replenished with cycloheximide containing medium, produced TPA only in minimal amounts. TPA activity of the culture medium was determined only in 1 experiment and the results show the same trend as TPA extraction experiments. Medium collected from the control explants on the 5th culture day produced 49 mm^2 of lysis on fibrin plates compared to 26 mm^2 from cycloheximide treated explants. Medium from explants, which were exposed to cycloheximide only for the 1st 2 days in culture and then allowed to recover, produced 69 mm^2 of lysis.

These results show that cycloheximide prevents the production of TPA by explants of the rat embryo tongue. This inhibition is more marked than that produced by hydrocortisone[5]. The inhibition by cycloheximide is reversible, consistent with the reversible influence of cycloheximide on protein synthesis[11]. The observed effects of cycloheximide and hydrocortisone show that it is possible to influence the cellular production of TPA. Histochemical studies have shown that cellular production and release of TPA is related to the stages of cellular maturation and degeneration[13]. Cells transformed by oncogenic virus produce a fibrinolytic agent, the production of which is reported to be blocked by cycloheximide, and did not parallel the increase in lysosomal enzymes[14]. Several authors have reported that cortisone delays the process of degeneration[15-17], and cycloheximide is reported to delay the collapse of epithelial cells and the progress of tissue regression in the castration induced involution of the prostate[18]. The observed effects of hydrocortisone and cycloheximide on explants of normal epithelial tissue support our contention[13] that the cellular production and release of plasminogen activator are mechanisms involved in the regulation of cell desquamation and tissue regression.



Concentrations of plasminogen activator in extracts from rat embryo tongue explants maintained in the presence or absence of cycloheximide (5 µg/ml). The data represent 2 individual experiments (-○-, -●-). Each point was derived by pooling explants from 3 culture dishes: ————, without cycloheximide, ------, cycloheximide present.

1 This study was supported by a USPHS grant (HE-05050) from the National Heart and Lung Institute.
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Improved method for preparation of microcrystalline chlorophyll a with *Anacystis nidulans* as a source[1]

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Summary. Because of the lack of chlorophyll b, blue-green algae are a better source of microcrystalline, high-purity chlorophyll a than the spinach leaves so far used. The preparation is easy and rapid, and the danger of chemical alteration of the chlorophyll is reduced.

High-purity, microcrystalline chlorophyll a (m-chl a) is needed for studies of the photoactivity of this substance[2,3]. It has now been found that blue-green algae are superior as a source of m-chl a to the spinach leaves that are generally used. The advantage consists in the absence of chlorophyll b from blue-greens, which radically facilitates purification. Moreover, at least one of these algae, namely *Anacystis nidulans*[4], can be grown easily, rapidly and axenically.

The method of extraction is derived from that of Strain et al.[5] The algae (strain L 1402-1 from the Culture Collection of Algae, Göttingen, BRD) were harvested, washed with 20% acetone and water, and extracted 3–5 min with metha-

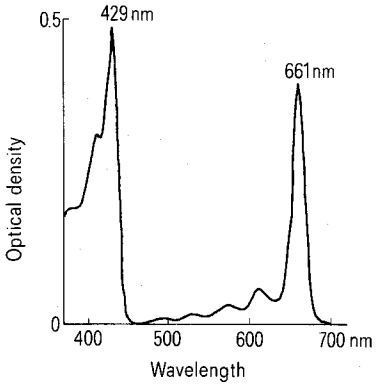

Fig. 1. Absorption spectrum of chlorophyll a in diethyl ether.

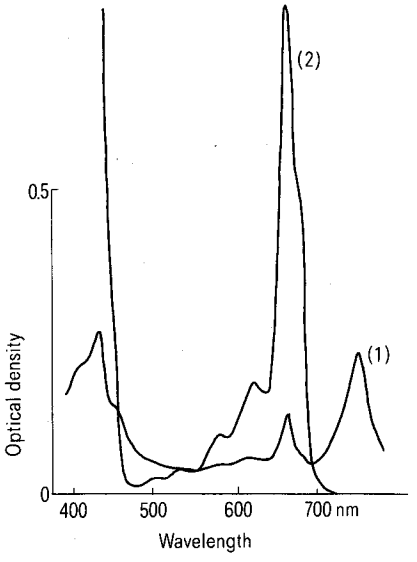

Fig. 2. (1) Absorption spectrum of suspension/solution of chlorophyll a in isooctane; (2) Same sample heated 10 min to 80 °C.

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 °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[5]. 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 °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 CrK_α powder pattern agreed with that obtained by Donnay[6]. Elemental analysis and IR spectography 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.

1 Acknowledgments. I thank Prof. E. Broda for his interest and Prof. H. Falk for valuable advice. I am also indebted to Mr E. Maday (IR spectography), Dr J. Seifert (X-ray) and Dr H. Zak (elemental analysis). The work was supported by the 'Fonds zur Förderung der wissenschaftlichen Forschung' of the Republic of Austria.

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Effects of selective removal of the salivary glands on taste bud cells in the vallate papilla of the rat[1]

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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[2,3]. 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[4,5] and subsequently mature into intermediate cells[6]. Light cells are aged intermediate cells close to leaving the system by desquamation[6]. 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[7,8], 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[8]. 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