with no indication of its secretion. This result suggests that c-AMP is perhaps involved at some stage in the biosynthesis of TSH rather than in its secretion. However, we must keep in mind that exogenic dibutyryl c-AMP is not identical to endogenic c-AMP.

Table 3 shows results of an experiment with separated thyrotropic cells which were incubated with PGE_2, and the TSH and c-AMP levels were measured. It was found that 10^{-5} M PGE_2 increased c-AMP level with no change in the content or secretion of TSH. This result provides further proof that a rise in c-AMP levels is not correlated with the level of TSH.

In conclusion, our data demonstrate that TRH causes a rise in content and secretion of TSH in separated thyrotropic cells and that the rise in accumulation and secretion of TSH, after incubation with different amounts of TRH is not dependent on the 3',5',c-AMP level. These results are further fortified by our findings that elevation of 3',5',c-AMP concentration in the thyrotropic cells during their incubation with PGE_2 is not directly linked with TSH production and release.

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Inhibition of plasminogen activator production in organ cultures by cycloheximide[1]

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Summary. The production of tissue plasminogen activator (TPA) in rat tongue organ cultures is strongly inhibited by low concentrations of the protein synthesis inhibitor cycloheximide. TPA production is fully resumed after the removal of cycloheximide from the culture medium.

Tissue plasminogen activator (TPA) which converts the proenzyme plasminogen into the fibrinolytically active protease plasmin and thus plays a major role in the processes of hemostasis and tissue repair, is widely distributed in mammalian tissues, but is particularly apparent in vascular endothelium, in various epithelia, and in blood leukocytes[4]. In explant cultures of rat tongue hydrocortisone strongly inhibits TPA production by epithelial cells, while typical lysosomal enzymes (betaglucuronidase, acid protease) remain essentially unaffected[5]. This finding has since been confirmed for various other cell types[6-9]. The inhibition of TPA synthesis by hydrocortisone is reminiscent of the reported inhibition by glucocorticoid hormones of protein synthesis in skin and muscle[10]. We now report that cycloheximide, a potent inhibitor of protein synthesis[11], also inhibits TPA production in the rat tongue.

Materials and methods. The organ culture procedures employed have been previously described[5]. Tongues from 16–20-day-old embryos of DUB/SDD rats were split along the midline and cultured in a medium consisting of Trowell T8 medium (Microbiological Associates, Bethesda, MD) buffered with 14 mM Hepes (General Biochemicals, Chagrin Falls, Ohio). No serum or antibiotics were added. Cycloheximide was obtained from Sigma Chemical Co., St. Louis, Missouri, and Upjohn Co., Kalamazoo, Mississippi. Appropriate solutions were prepared in Hank's BSS and sterilized by filtration (Falcon 0.22 µm membrane filter). Varying concentrations were added to the culture medium in 50 µl volumes. An equal volume of Hank's BSS was added to controls.

TPA activity was determined by extraction of pooled explants from 3 or 4 culture dishes with 2 M potassium thiocyanate followed by acid precipitation[12]. Activities were determined on plasminogen-rich fibrin plates. Concentrations were obtained by interpolation on a standard dilution curve[12] and reported in units per g wet wt or per mg protein (determined by the method of Lowry). The TPA released into the culture medium was also determined on fibrin plates. A solution of cycloheximide alone (5 µg/ml) was tested on fibrin plates in combination with a TPA standard (2 units/ml) and proved to have no influence on the mechanism of plasminogen activation. All determinations of fibrinolytic activity were carried out also on plasminogen-free fibrin and no evidence of nonspecific proteolysis was found.

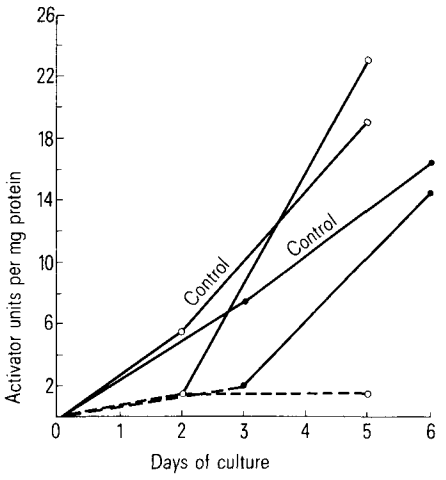
Results and discussion. Data from 3 experiments (designated as A, B and C) are summarized in the table to show the range of individual values obtained as well as the time course of changes in TPA concentration. Controls showed the previously reported progressive increase in extractable TPA in tongue explants maintained in normal culture medium. In explants maintained in the presence of 5 µg of cycloheximide this rapid and marked increase of TPA activity was considerably inhibited. In 9 separate determinations cycloheximide (5 µg/ml) treated explants were found to contain only 15±10% of the TPA concentration of controls, regardless of the time point during the 6-day

Inhibition of plasminogen activator production in explants of embryonic rat tongue. The data represent 3 individual experiments (A, B, C). Each result was derived by pooling explants from 3 culture dishes. Extracts were prepared with 2 M potassium thiocyanate followed by acid precipitation

Experiment	Days in culture	Concentrations of TPA (units/g wet wt)		Inhibition (%)
		Controls	Cycloheximide (5 µg/ml)	
A	0	10	-	-
B	0	2	-	-
C	0	3	-	-
A	3	580	38	93
B	3	328	49	85
C	2	100	18	82
A	6	1150	17	99

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.

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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-