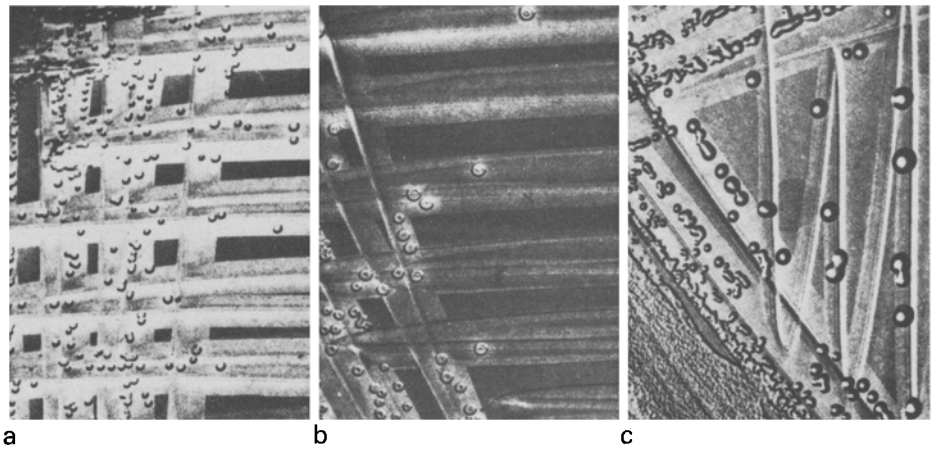

Morphologie des colonies de pneumocoque (× 2) sur gélose au sang humain après 24 h d'incubation à 37 °C. *a* en aérobiose, *b* en anaérobiose relative, *c* en anaérobiose stricte (avec adjonction de CO_2).

dans le 93% des cas la croissance de colonies de diamètre sensiblement plus élevé, bien vertes, pratiquement toujours ombiliquées. L'hémolyse *α* est large. Jamais les colonies n'ont été plus petites qu'en aérobiose. L'incubation à 37 °C avec adjonction de H_2+CO_2 (anaérobiose stricte) permet dans le 70% des cas la croissance de colonies de diamètre encore plus élevé qu'en anaérobiose relative, gris-verdâtre, non ombiliquées puisque la croissance est le plus souvent muqueuse. L'hémolyse *α* est réduite. Jamais les colonies n'ont été plus petites qu'en anaérobiose relative.

Quant aux résultats de la numération de germes, ils sont donnés dans le tableau. L'origine des souches ne semble jouer aucun rôle; de même, ce ne sont pas obligatoirement les souches qui ont moins bien - respectivement mieux - poussé en anaérobiose relative qu'en aérobiose qui poussent moins bien - respectivement mieux - en anaérobiose stricte qu'en anaérobiose relative. La différence de croissance varie en moyenne dans un rapport de 1 à 3 fois si l'on compare l'aérobiose à l'anaérobiose relative, et de 1 à 1,5 fois si l'on compare l'anaérobiose relative à l'anaérobiose stricte. Dans un cas les géloses sont restées stériles en aérobiose.

Nous avons fait la preuve que l'aérobiose n'est pas à recommander. Les pneumocoques n'y poussent en effet que peu nombreux, et si petits qu'ils ne sont parfois plus reconnaissables. L'anaérobiose relative était, et est encore, le mode d'incubation traditionnellement utilisé puisqu'il permet, à peu de frais, une croissance améliorée des pneumocoques. Pourtant, en 1976, Howden[2] fait remarquer que le 52,3% des souches de pneumocoque qu'il a isolées à partir de frottis des voies aériennes supérieures étaient anaérobies strictes. Ayant, pour ce travail, isolé nos souches en anaérobiose relative, il ne nous a pas été permis de mettre en évidence des souches anaérobies strictes; nous nous souvenons cependant d'en avoir isolé, hors étude, dans des pus de sinus et une plaie appendiculaire notamment. L'anaérobiose stricte offre donc le double avantage de permettre une croissance encore améliorée des souches aérobies-anaérobies facultatives[2,4], et l'isolation des souches anaérobies strictes[2,5] qui sont sans doute plus nombreuses qu'on ne le pensait jusqu'à ces dernières années.

Nos travaux, en y ajoutant la notion de comptage, corroborent la recommandation de Howden[2], suivi déjà par Yatabe et al.[5] qui relevait la nécessité d'introduire systématiquement l'anaérobiose stricte pour l'isolation des pneumocoques. Pourtant nous aimerions ne pas négliger l'anaérobiose relative couramment employée, cela pour ne pas laisser passer éventuellement les pneumocoques muqueux. Nous avons démontré en effet, et cela n'avait pas encore été publié à notre connaissance, que les pneumocoques muqueux ne poussent qu'en très petit nombre en anaérobiose stricte. On emploiera donc parallèlement les 2 modes d'incubation toutes les fois que l'on suspectera une infection à pneumocoque.

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Correlation between 3′,5′, c-AMP levels and thyrotropin in separated rat pituitary thyrotropic cells

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Summary. The correlation between 3′,5′, c-AMP levels, TSH content and secretion of separated thyrotropic cells was studied. Incubation of the separated cells with 1, 10 and 100 ng of TRH does not change the 3′,5′,c-AMP levels, despite the significant rises of the TSH level. Dibutyryl c-AMP causes rise in TSH content, with no indication of its secretion. PGE_2 10^{-5} increased 3′,5′,c-AMP levels with no change in the content or secretion of TSH in separated thyrotropic cells.

Among the numerous questions regarding the regulation of biosynthesis and secretion of thyrotropic stimulating hormone (TSH) from the anterior pituitary gland, there is also the much criticized and long debated problem of whether 3′,5′ cyclic AMP and prostaglandins are involved in the above processes, and whether, as in the case of the thyroid gland, there is a correlation between the levels of 3′,5′, c-AMP and hormonal regulation[1,2].

Table 1. Effect of TRH on separated thyrotropic cells. Relationship between 3′,5′, c-AMP levels, TSH accumulation and release (means ± SEM)

Treatment/cells	TSH accumulation ($\mu g/1.5 \times 10^6$ cells)	TSH release ($\mu g/1.5 \times 10^6$ cells)	3′,5′, c-AMP levels (pmoles/1.5×10^6 cells)
Control 2 h incubation at 37 °C	14 ± 1.3	86 ± 5.3	3.23 ± 0.99
TRH 1 ng 2 h incubation at 37 °C	22 ± 1.8**	293 ± 17.4*	3.66 ± 0.23
TRH 10 ng 2 h incubation at 37 °C	37 ± 2.6*	403 ± 26.7*	3.06 ± 0.17
TRH 100 ng 2 h incubation at 37 °C	63 ± 5.5*	1636 ± 101.5*	3.71 ± 0.29

* $p = 0.001$; ** $p = 0.01$.

Table 2. Effect of 0.3 mM DB-c-AMP on TSH accumulation and release by separated thyrotropic cells (means ± SEM)

Treatment/cells	TSH accumulation ($\mu g/1.5 \times 10^6$ cells)	TSH release ($\mu g/1.5 \times 10^6$ cells)
Control 2 h incubation at 37 °C	12 ± 1.0	75 ± 5.7
0.3 mM DB-c-AMP 2 h incubation at 37 °C	31 ± 0.8*	70 ± 4.9

* $p = 0.001$.

Studies have indicated cyclic AMP as mediator of TSH secretion by the anterior pituitary gland[3,4]. Although many different functions have been proposed for the prostglandins, until recently there has been relatively little consideration given to the possible roles of these ubiquitous fatty acids in neuroendocrine regulation of anterior pituitary secretion. Vale et al.[5] have shown that TSH release from cultured anterior pituitary cells is increased by the addition of PGE to the incubation medium. Similarly, it has been reported that PGE will increase TSH release by hemipituitaries incubated in vitro[6]. In contrast, Brown and Hedge[7] reported that the prostaglandins do not increase the release of TSH in vitro. We reported in 1973[8] that we had found no elevation in the levels of c-AMP in the pituitary gland after incubation with various amounts of TRH, despite the fact that TRH induced the secretion of TSH. On the other hand, we found a large and significant elevation in c-AMP levels, after incubation with PGE_2, but incubation of the gland with PGE_2 had no influence on the TSH secretion.

Until now, in resulting work from using preparations of whole glands in vivo and in vitro, we decided to try and check these results on isolated and separated thyrotropic cells, in order to obtain a clearer picture.

Materials and methods. Male albino rats of the Hebrew University Sabra strain, 40–45 days old weighing 80–100 g, were used. All rats were fed standard laboratory pellets and water ad libitum. The animals were killed by decapitation, their pituitaries were removed quickly, and the anterior lobes were transferred into Earle's medium pH 7.2 without Mg^{+2} or Ca^{+2}. The gland was subjected to trypsinization with 0.1% trypsin solution (Difco 1:250) contained 0.1% bovine serum albumin and buffered with 1.5% $NaHCO_3$ to pH 7.2. After trypsinization, the cells were concentrated by centrifugation, washed twice with Earle's solution and resuspensed in M-199 solution containing 10% foetal calf serum.

Thyrotropic cells were separated by the technique of binding these cells to nylon fibres as we reported previously[9]. The cultures we obtained contained 80–85% thyrotropic cells. The cells were used immediately after separation, without growing them in culture media. In the experiments, the cells were incubated with different treatments in a M-199 medium. The calculation of the results was made per 1.5 million thyrotropic cells (700 µg protein).

Thyroid stimulating hormone (TSH) levels were determined by radioimmunoassay using reagents supplied by the Rat Pituitary Program of the National Institute of Arthritis, Metabolism and Digestive Diseases (NIAMDD). Results were expressed in terms of the NIH RP-1 rat reference preparations.

3′,5′, c-AMP levels were determined by radioimmunoassay using New England Nuclear cyclic AMP (^{125}I) RIA Kit (NEN, 549 Albany St., Boston, Ma. USA). The TRH was a generous gift from Farbwerke Hoechst AG, Frankfurt; PGE_2 were kindly furnished by Dr John Pike of The UpJohn Company, Kalamazoo Mich. Rat TSH and anti-rat TSH were obtained from Dr A. Parlow, NIAMDD.

Results and discussion. Table 1 shows that incubation with 1, 10 and 100 ng of TRH does not change the 3′,5′, c-AMP levels, despite the significant rises of the TSH level. This finding cannot refute the possible role of c-AMP in the regulation of secretion and biosynthesis of TSH, as it is possible that the increments in c-AMP levels were so small, that they were undetectable, or the rate of metabolism of c-AMP is equal to its rate of biosynthesis. But it may also be that the difference in c-AMP levels is not related to the biosynthesis and secretion of TSH. However, when the separated cells were incubated with dibutyryl c-AMP (table 2), we obtained a significant rise in TSH content,

Table 3. Effect of PGE_2 on separated thyrotropic cells: Relationship between 3′,5′, c-AMP levels, TSH accumulation and release (means ± SEM)

Treatment/cells	TSH accumulation ($\mu g/1.5 \times 10^6$ cells)	TSH release ($\mu g/1.5 \times 10^6$ cells)	3′,5′, c-AMP levels (pmoles/1.5×10^6 cells)
Control 1 h incubation at 37 °C	14 ± 1.1	70 ± 6.4	3.93 ± 0.32
PGE_2 10^{-5}M 1 h incubation at 37 °C	12 ± 0.9	74 ± 7.0	9.3 ± 0.88*

* $p = 0.05$.

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