Zusammenfassung. Durch Fusion von Mikroplasmodien des Schleimpilzes *Physarum polycephalum* wird der Mitosecyclus ihrer Kerne synchronisiert. Durch künstlich verzögerte Fusion können Tochterkerne einer soeben erfolgten Mitose zur Teilnahme an der ersten synchronen Mitose nach der Fusion gezwungen werden, bevor die morphologische Rekonstitution und die Duplikation ihrer DNS vollendet sind.

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Modifications of Thyroid Activity by Melatonin

In a previous work we have reported that the administration of a pineal extract is capable of preventing thiouracil goitre in rats[1]. These results are in keeping with those of ARON et al.[2], who recently observed exophthalmos in turtles after pinealectomy. On the contrary, YAMADA et al.[3] did not find after treatment with thiouracil any significative weight difference in thyroids from pinealectomized and control rats.

These results moved us to investigate the influence on the thyroid of Melatonin, which is N-acetyl-5-methoxytryptamine, a lightening substance, isolated in the pineal body by LERNER et al.[4].

Material and Methods. These experiments were carried out on albino Wistar male rats (mean weight 150 g), fed during the experimental period of ten days with Purina Fox. Melatonin was supplied by Regis Chem. Co., California, eluted in hydroalcoholic solution (50% water and 50% ethyl alcohol 90°C), and injected subcutaneously.

The animals were divided into six groups: the first group (A) were the controls; the second group (B) drank *ad libitum* a suspension of 0.3 mg/ml of methylthiouracil (MTU); the third group (C) were injected every day with 0.25 ml of hydroalcoholic solution; the fourth group (D) drank *ad libitum* a suspension of 0.3 mg/ml of MTU and were injected every day with 0.25 ml of hydroalcoholic solution; the fifth group (E) received subcutaneously every day 150 γ of melatonin in 0.25 ml of hydroalcoholic solution; the sixth group (F) drank *ad libitum* a suspension of 0.3 mg/ml of MTU and were injected every day with 150 γ of melatonin in 0.25 ml of hydroalcoholic solution. On the 10th day, each rat was given 0.8 μC of carrier-free radioiodine.

After 24 h, the rats were killed and the thyroid glands were immediately removed and weighed on a torsion balance. Thyroid radioactivity was then calculated as % of the dose formerly injected (a standard was specifically prepared).

Histological studies were made on the thyroid tissue stained with hematoxylin-eosin and hematoxylin-orange-phospho-molybdic acid-aniline blue. The cellular height was measured with the help of a Leitz Ortholux Microscope. The cells magnified 2000 times in diameter were drawn on millimeter paper; an average of 100 cells were measured for every tissue slide. Standard deviation of results was evaluated following the formula.

$$\sigma(m) = \sqrt{\frac{Sx^2}{(n-1)\,n}}$$

Parameter t was calculated as follows

$$t = \frac{M_1 - M_2}{\sigma \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \quad \text{where } \sigma = \sqrt{\frac{Sx^2_1 + Sx^2_2}{(n_1 - 1) + (n_2 - 1)}}$$

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[2] E. ARON, C. COMBESCOT, J. DEMARET, and L. GUYON, C. R. Acad. Sci. *251*, 1914 (1960).
[3] T. YAMADA, Endocrinology *69*, 706 (1961).
[4] A. B. LERNER, J. D. CASE, and R. V. HEINZELMAN, J. Amer. chem. Soc. *81*, 6084 (1959).

Group and number of animals		Treatment		Thyroid weight mg % of body weight	Cell height in μ	Thyroid ^{131}I uptake % of the dose
A	(10)	Purina Fox diet, H_2O		14.9 ± 1.5	5.8 ± 0.16	42.2 ± 3.4
B	(10)	Purina Fox diet, H_2O + MTU		27.5 ± 1	13 ± 0.11	3.8 ± 0.02
C	(10)	Purina Fox diet, H_2O, hydroalcoholic solution		12.3 ± 1.7	7.6 ± 0.14	35 ± 4.4
D	(10)	Purina Fox diet, H_2O, MTU, hydroalcoholic solution		20.7 ± 1.3	13.5 ± 0.39	11.7 ± 4.1
E	(10)	Purina Fox diet, H_2O, melatonin in hydroalcoholic solution		11.4 ± 0.21	3 ± 0.95	2 ± 0.2
F	(10)	Purina Fox diet, H_2O, MTU, melatonin in hydroalcoholic solution		19.3 ± 1.7	8.7 ± 0.19	3.9 ± 0.03
		t	A–C	1.13	2.8	1.16
			A–E	2.23	15	10.8
			B–D	4.07	0.88	3.3
			B–F	3.56	9.5	13.2
			C–E	0.48	29	7.27
			D–F	1.1	10.9	4.12

theoric t ($P = 0.05$) = 2.3; ($P = 0.01$) = 3.35; ($P = 0.001$) = 5.04

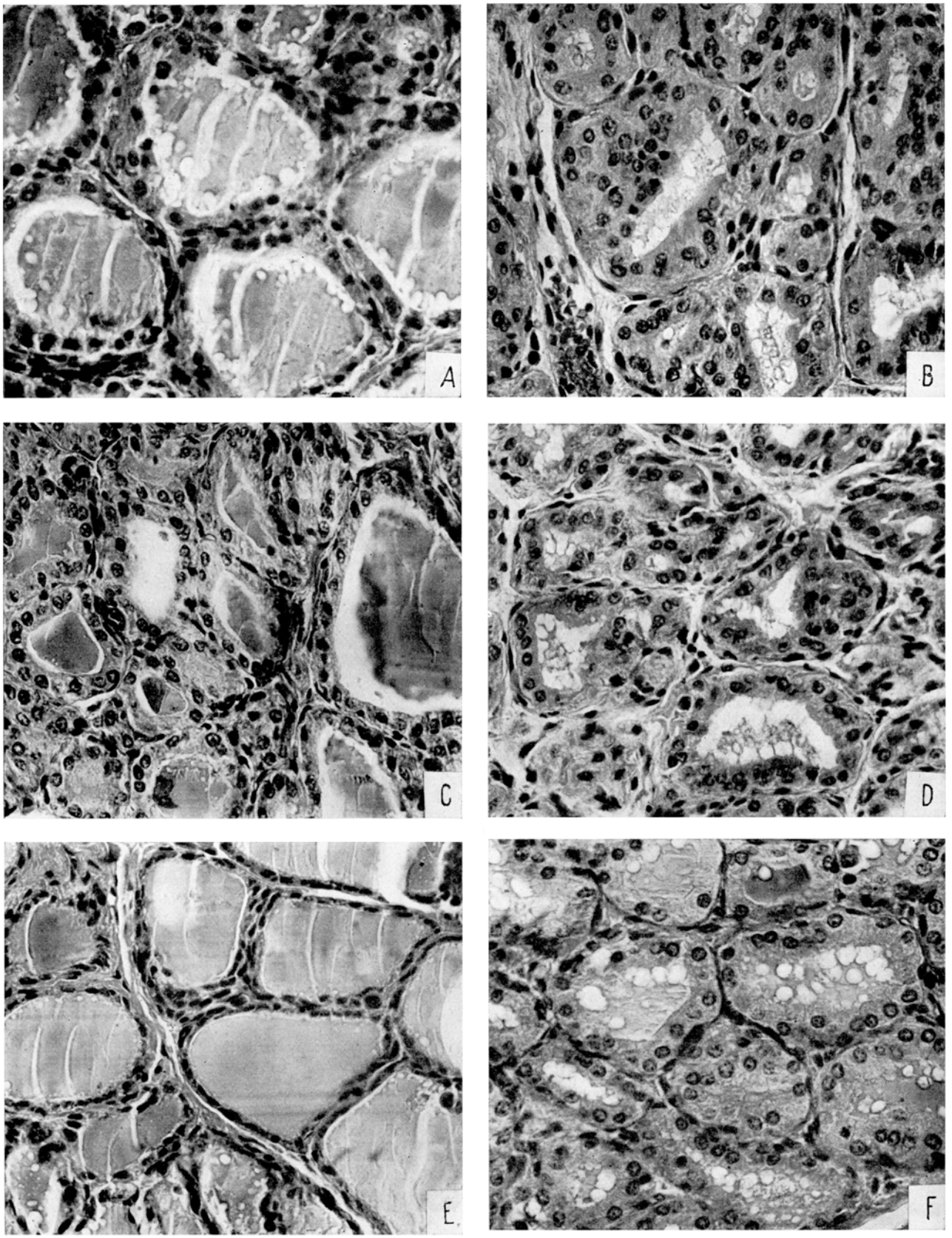

Fig. 1. Histological picture of *A* Thyroid of normal rats (cell height 5.8 μ); *B* Thyroid of rats treated with MTU (cell height 13 μ); *C* Thyroid of rats treated with hydroalcoholic solution (cell height 7.6 μ); *D* Thyroid of rats treated with hydroalcoholic solution and MTU (cell height 13.5 μ); *E* Thyroid of rats treated with Melatonin (cell height 3 μ); *F* Thyroid of rats treated with MTU and Melatonin (cell height 8.7 μ). Photomicrography 410 ×, fixation Bouin, Hematoxylin-oesin.

Results. The results are given in the Table. Thyroid weight in animals treated with Melatonin (group E) was markedly lower (23.5%) than in the control group (A) and the difference was statistically significant ($t = 2.23$). In the animals treated with MTU and Melatonin (F), thyroid weight was lower (29.7%) than in animals which received only MTU (B) and the statistical difference was very significant ($t = 3.56$).

In animals treated with hydroalcoholic solution (C) thyroid weight was lower than in the control group (A) but the difference was not statistically significant ($t = 1.13$). On the contrary, in the group treated with MTU and hydroalcoholic solution (D) the thyroid weight was markedly lower (24.7%) than in the group treated by MTU only (B) and the difference was statistically significant ($t = 4.07$). Since the difference between groups treated only by hydroalcoholic solution and groups treated by a hydroalcoholic solution of Melatonin is low and not statistically significant, it is reasonable to think that the weight difference between C and A is mainly due to the influence of alcohol.

Histologically the differences are more evident (Figure). The height of the cells in the animals treated with Melatonin (E) and in those treated with Melatonin and MTU (F) was markedly lower than in the control group (A) and in the group treated with MTU (B). The difference were more significant respectively ($t = 15$, $t = 9.5$). There was no difference between animals treated with hydroalcoholic solution and MTU (D) and animals treated with MTU only (B).

The discrepancy observed between the behaviour of gland weight and cell height could be explained by supposing that the weight modification induced by alcohol is due to reduction of the thyroid colloid or of the thyroid vascularity.

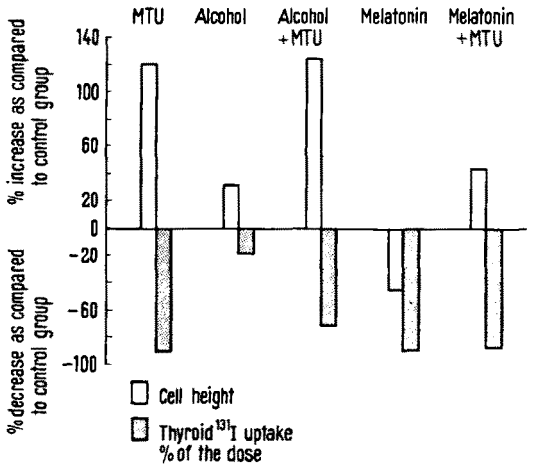

Fig. 2. Cell height (evaluated on 100 cells) of follicular thyroid epithelium and ^{131}I uptake changes in Melatonin, Alcohol, MTU, Alcohol + MTU, Melatonin, Melatonin + MTU treated rats.

The ^{131}I uptake was definitely lower (95.2%) in animals treated with Melatonin (E) than in the control group (A) and the difference was statistically significant ($t = 10.8$). As was to be expected, the ^{131}I uptake was reduced in animals treated with MTU (90.9%) (B), but the administration of Melatonin did not produce any further reduction of ^{131}I uptake (F). In animals treated with hydroalcoholic solution (C), the ^{131}I uptake was slightly lower (16%) than in the control group (A), but the difference was not statistically significant ($t = 1.16$); in animals which received hydroalcoholic solution and MTU (D) the ^{131}I uptake was lower (74%) than in control group (A), but the reduction was less than in animals who received MTU only (B). The value of these results is difficult to explain. The experience was repeated with a solution of Melatonin in glycerol. This solution decreased thyroidal ^{131}I uptake only by 43% compared with the controls.

Discussion. According to our results Melatonin has an inhibitory effect on thyroid function. This substance is able to prevent thyroid hyperplasia by MTU, and to reduce the thyroidal ^{131}I uptake. These results suggest that the modifications produced by a pineal extract (De Luca et al.[1]) may be due to Melatonin.

Rats treated with alcohol showed a decrease in thyroid weight, those treated with MTU and alcohol showed a smaller increase of thyroid weight than those treated with MTU only. On the other hand, the height of thyroid cells and ^{131}I uptake were not affected by the administration of alcohol. The significance of these modifications is not clear. Aschkenasy and Guerin[5] have observed in rats a moderate effect of alcohol on thyroid function.

On the basis of our results, we cannot entirely explain the influence of Melatonin on thyroid activity. As this substance prevents the increase of thyroid weight in animals treated with thiouracil, it is reasonable to think that melatonin could have an inhibitory effect on TSH secretion or on the action of this hormone on thyroid gland. Both of these hypotheses are now under further investigation in our Department.

Riassunto. Gli autori hanno studiato la funzione tiroidea in ratti trattati con soluzione idroalcoolica di Melatonina ed hanno osservato che negli animali così trattati il peso della ghandola, l'altezza delle cellule follicolari, e la captazione del radioiodio si riduceva.

La Melatonina ha anche inibito la formazione della iperplasia tiroidea da tiouracile.

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Istituto di Patologia Speciale Medica e Metodologia Clinica dell'Università di Roma (Italy), August 6, 1962.

[5] P. Aschkenasy, P. Lelu, and M. T. Guerin, C. R. Soc. Biol. *154*, 1409 (1960).

Ribonuclease- und Trypsinwirkung auf isolierte Nebennierenmark-Granula

Die Brenzcatechinamine – Adrenalin und Noradrenalin – werden nach den heutigen Vorstellungen in den Nebennierenmarkgranula, cytoplasmatischen Organellen der chromaffinen Zelle, als Komplex mit ATP und Eiweiss gespeichert, wobei auf 1 Molekül ATP ungefähr 4 Moleküle Amine entfallen (Blaschko, Born, D'Iorio und Eade[1], Falk, Hillarp und Högberg[2], Schü-

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[2] B. Falck, N. A. Hillarp und B. Högberg, Acta physiol. scand. *36*, 306 (1956).