reduces the number of viable 3T3-MVS-H and RTC cells; it kills all the S 180-A cells. As shows in Table II (exp. 1) the toxicity of FUDR-RSA cannot be due to FUDR merely adsorbed onto RSA. The action of FUDR-RSA is abolished by the addition of thymidine (Table II, exp. 2); this indicates that the conjugate acts by releasing FUDR.

At the concentration which completely inhibits the proliferation of the neoplastic cells, FUDR-RSA does not affect the non-dividing macrophages, although it seems very likely that these cells have a much higher protein uptake. In fact macrophages are many times more sensitive than the neoplastic cells to the AMA-RSA conjugate.

The present results indicate that FUDR coupled with albumin is released in active form after penetration of the conjugate into the cells. A different result was obtained by coupling two other antineoplastic drugs, methotrexate and adriamycin, to albumin. The methotrexate conjugate was completely ineffective on the neoplastic cells and on macrophages, whereas the adriamycin conjugate displayed only a very slight action as compared to the strong effect produced by equivalent amounts of the free drug.

In future experiments we intend to test the action of FUDR-RSA on the in vivo-growth of those tumours whose cells take up proteins to a high degree. It is worth noting that FUDR-RSA has a low toxicity; injected i.p. at a dose of 1 mg/10 g body weight (the maximum tested) it does not kill mice, while the LD_{50} (i.p.) of AMA-RSA for the mouse is only 1.5 µg/10 g body weight.

Since only about 1% of input FUDR is conjugated to RSA by our ECDI procedure, some difficulties arise to obtain the amount of conjugate necessary for the in vivo experiments, which should be postponed until a search is made for a more effective method of conjugation[19].

Riassunto. La 5-fluorodeossiuridina, unita covalentemente alla albumina di coniglio, è liberata in forma attiva dopo penetrazione del coniugato dentro le cellule.

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Enhancement of Cerebral Noradrenaline Turnover by Thyrotropin-Releasing Hormone: Evidence by Fluorescence Histochemistry

Thyrotropin-releasing hormone (TRH) has been claimed to improve mental depression[1,2]. TRH potentiates the behavioral effects of L-DOPA plus pargyline in mice[3]; this action seems independent of the release of thyroid-stimulating hormone (TSH), because the potentiation occurs in hypophysectomized mice, as well as in normal mice, and may be connected with a direct potentiation of catecholamine systems. Biochemical findings have been reported[4] indicating that TRH enhances the turnover of noradrenaline in the brain of rats, enhancement observed in thyroidectomized rats as well as in normal rats.

We investigated morphologically the action of TRH on the monoamine neurons of the rat brain. 30 male albino rats of Wistar origin, 200–250 g, were used. The animals were injected i.p. with 300 mg/kg α-methyl-p-tyrosine (α M_PT), TRH + α M_PT, TRH alone and saline (controls). α M_PT was administered 4 h before sacrifice and TRH (20,30 or 40 mg/kg) $^1/_2$, 1, 2 or 3 h before α M_PT. The brains cut perpendicularly in 5 frontal sections were frozen in propane cooled in liquid nitrogen, freeze-dried and treated with formaldehyde, according to the method of FALCK and HILLARP[5]. Sections 10 µm of all these brains were mounted semi-serially (1/10, 1/20); the observation of the sections by the fluorescence microscope under standard conditions permitted us to obtain a subjective morphological appreciation of the changes in the fluorescence intensity of monoaminergic neurons at various cerebral areas.

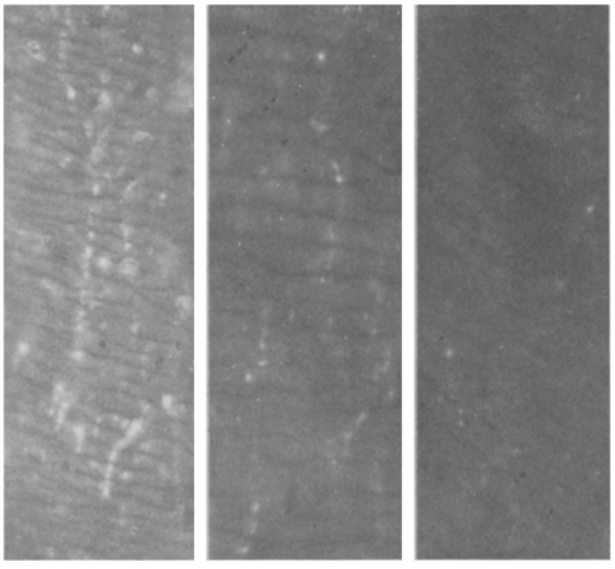
After administration of α M_PT a marked decrease of the green fluorescence of the dopamine and noradrenaline cell bodies (locus niger and locus coeruleus + lateral pontobulbar nuclei, respectively) occurred. The green fluorescence of the dopamine terminals (neostriatum) and that of noradrenaline terminals of hypothalamus and cerebral cortex was also strongly diminished (Figure b). No changes in the yellow fluorescence of 5-hydroxytryptamine neurons was observed.

TRH in doses of 20–40 mg/kg administered $^1/_2$, 2, 3 h before αM_PT accentuated the decrease of green fluorescence in the noradrenaline terminals of cerebral cortex (Figure). In the hypothalamic terminals, however, the αM_PT-induced decrease of noradrenaline fluorescence was slightly accentuated after 40 mg/kg TRH administered $^1/_2$ or 1 h before αM_PT, but did not change after lower doses of TRH, and if TRH was administered 2 or 3 h before αM_PT.

The αM_PT-induced decrease of the noradrenaline green fluorescence at locus coeruleus cell bodies, and of the dopamine green fluorescence at the locus niger cell bodies and neostriatal terminals, was not influenced by TRH.

The fact that TRH accentuated the αM_PT-induced decrease of the green fluorescence of cortical and hypothalamic noradrenaline terminals suggests that this tripeptide enhanced noradrenaline release. Since no

[1] A. J. KASTIN, R. H. EHRESING, D. S. SCHALCH and S. M. ANDERSON, Lancet *2*, 740 (1972).
[2] A. J. PRANGE JR., I. C. WILSON, P. P. LARA and L. B. ALLTOP, Lancet *2*, 999 (1972).
[3] N. P. PLOTNIKOFF, A. J. PRANGE JR., G. R. BREESE, M. S. ANDERSON and I. C. WILSON, Science *178*, 417 (1972).
[4] H. H. KELLER, G. BARTHOLINI and A. PLETSCHER, Nature, Lond., in press.
[5] B. FALCK, N. A. HILLARP, G. THIEME and A. TORP, J. Histochem. Cytochem. *10*, 348 (1962).



a) b) c)

Superficial layer of frontal cortex of rat brain. a) Control: green fluorescence of noradrenaline terminals. b) After αM_PT: decrease of the fluorescence of noradrenaline terminals. c) After TRH + αM_PT: the αM_PT-induced decrease of fluorescence is accentuated. ×480.

change in noradrenaline fluorescence was observed after administration of TRH alone, it must be assumed that the synthesis compensated for the release of the amine. Therefore the turnover of noradrenaline is enhanced by TRH. This effect was more evident in cortical than hypothalamic noradrenaline terminals, but this difference may be related to the volume of the noradrenaline granules being much smaller in the cortex than in the hypothalamus.

In conclusion, TRH probably causes an activation of noradrenaline neurons in the brain, and this effect might be connected with the antidepressant action of the tripeptide in man.

Zusammenfassung. Durch Histofluoreszenz-Methode für Monoamine wurde nachgewiesen, dass die Abnahme der grünen Fluoreszenz in den Noradrenalin-Endungen des Cortex und Hypothalamus im Rattenhirn durch α-Methyl-Paratyrosin verstärkt wird, was vermuten lässt, dass dieses Tripeptid die Freisetzung und Umkehrung des Noradrenalin erhöht.

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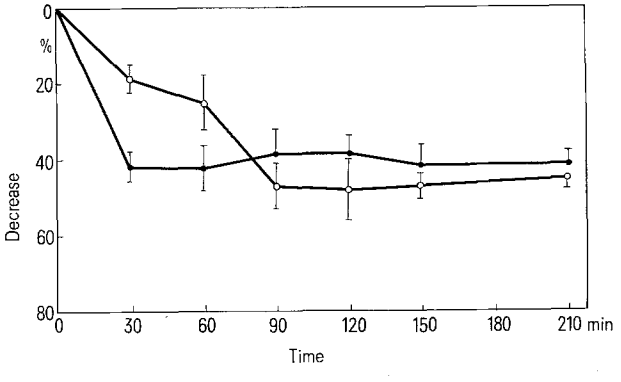
Effects of Cannabis Smoking in Blood Lactic Acid and Glucose in Humans

The study of the effect of cannabis on catecholamine concentration and metabolism has been the subject of several investigations. Thus it has been reported[1–3] that administration of hashish, marihuana or pure tetrahydrocannabinol produces considerable changes in the concentration of catecholamines in the brain. While others[4], though not observing similar changes, have reported an increased turnover. A central action can, therefore, be ascribed to cannabis with respect to biogenic amines[5]. On the other hand, it is well documented[6–8] that hashish smokers show an increased appetite especially for sweet foods during the recovery time. This phenomenon has been correlated to changes of the concentration of noradrenaline in the brain[9].



Decrease of lactic acid after hashish smoking during a period of 210 min. Each point is the mean from 5 subjects. ○—○, chronic smokers; ●—●, naives. The results are given as percentages of the value obtained before smoking.

To investigate whether changes in catecholamines in the periphery are also involved in this phenomenon, the effect of hashish smoking on blood glucose was studied with the hope that changes of the catecholamine would be reflected on the level of blood glucose. Blood lactic acid was also measured.

Materials. Blood was withdrawn before the initiation of the experiment, and data obtained from its analysis were used as reference values to estimate differences produced by smoking. Test blood samples were obtained at 30 min intervals for a period of $3^1/_2$ h, except the last sample which was taken at 1 h period. During the experiment the subjects were kept in a room specially prepared for this purpose. The sera were analyzed on the day of the experiment for lactic acid and glucose.

Two groups of subjects have been used in the present experiments. One group consisted of 5 chronic smokers with experience from 25 to 35 years, and the other group

[1] D. Holtzman, R. A. Lovell, H. Jaffe and D. X. Freedman, Science *163*, 1464 (1969).
[2] J. Constantinidis and C. J. Miras, Psychopharmacologia *22*, 80 (1971).
[3] C. J. Miras, T. A. Kephalas and D. P. Papadakis, Bull. Narcotics *23*, 33 (1971).
[4] L. Maître, M. Staehelin and H. J. Bein, Agents Actions *1*, 136 (1970).
[5] R. Dagirmanjian and H. C. Hodge, Agents Actions *1*, 46 (1970).
[6] C. J. Miras, *Some Aspects on Cannabis Actions in: Hashish, Its Chemistry and Pharmacology*. Ciba Foundation (J. A. Churchill, London 1965), p. 37.
[7] C. J. Miras, in *Drugs and Yuth* (Ch. C. Thomas, New York 1969), p. 191.
[8] R. C. Pillard, New Engl. J. Med. *283*, 294 (1970).
[9] S. P. Grossman, Am. J. Physiol. *202*, 872 (1962). – S. R. Sommer, D. Novin and M. Levin, Science *156*, 883 (1967).