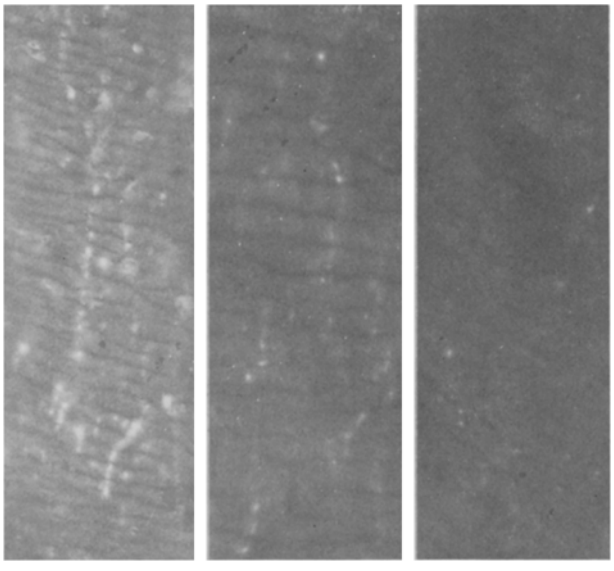

a) b) c)

Superficial layer of frontal cortex of rat brain. a) Control: green fluorescence of noradrenaline terminals. b) After $\alpha M_PT$: decrease of the fluorescence of noradrenaline terminals. c) After TRH + $\alpha M_PT$: the $\alpha M_PT$-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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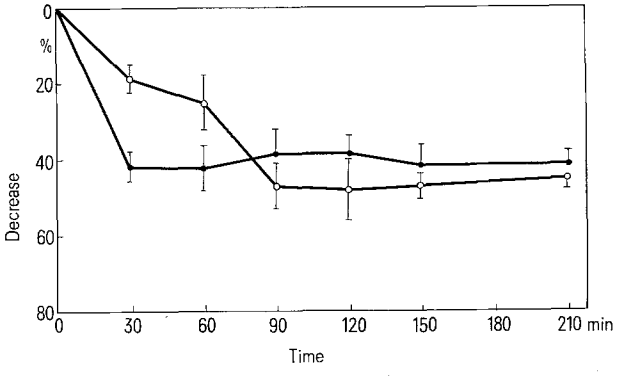
## Effects of Cannabis Smoking in Blood Lactic Acid and Glucose in Humans

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

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of 5 who had not smoked hashish for the last 3 years and had tested it 5 or 6 times in their life-time. This group was, therefore, considered as naives. All subjects were healthy volunteer tobacco smokers of ages ranging from 45 to 55 years, and they were fasted for 20 h before the experiment.

Hashish, 2 g from our own cultivation [(tetrahydrocannabinol (THC) 1.3%, cannabidiol (CBD) 0.4%, cannabinol (CBN) 1.1%)] was mixed with tobacco and placed half-way from its end of the cigarette. The duration of smoking was about 10 min.

*Methods of analyses.* Analysis of hashish extract. The sample of hashish smoked was analyzed by the method described by MIRAS[6] and it was found to contain THC, 1.3%, CBD 0.4% and CBN 1.1%.

Lactic acid. The enzymatic method described by HADJIIOANNOU et al.[10] was used to determine serum lactic acid. For this purpose the automatic reaction rate unit of Sargent was replaced by a Gilford spectrophotometer model 204. The instrument was calibrated so that the ratio of the concentrations of lactic acid/optical densities was independent of NAD and LDH. This ratio was linear in the range of 4 up to 24 mg of L-lactic acid per 100 ml of serum. The enzyme used was LDH (Sigma Type I from rabbit muscle). The same method was used to study the effect of THC and/or crude hashish (extracts) on the activity of pure LDH. The assay mixture contained in 3.2 ml and the tested materials added in alcohol solutions in a volume of 0.1 ml. The same volume of ethyl alcohol was added in the blank sample.

The hashish was extracted twice with petroleum ether for 2 h and an extraction containing several cannabinoids, corresponding to about 10% (w/w) of the hashish used was obtained. 5 extracts corresponding to 10, 25, 50, 75 and 100 mg of hashish respectively were evaporated to dryness, dissolved in 0.1 ml ethanol and each examined for their effects on the activity of pure LDH. It was observed that a slight turbidity of the incubation solution by using extracts corresponding to 50, 75 and 100 mg of hashish did not interfere with the measurements. By examining the effects of pure THC in concentrations up to $3.8 \times 10^{-7}$ *M* on the activity of pure LDH, no turbidity was observed.

Glucose. Glucose was determined by automatic method in an AII Technicon auto-analyzer by a colorimetric method, based on neocuproin-copper reaction[11].

*Results.* The effect of hashish smoking on the concentration of serum lactic acid is shown in the Figure. Each point of the curve is the mean of the values obtained from the 5 samples of each group. It can be seen that in both groups examined lactic acid concentrations decreased within 1 h of smoking. The rate of decrease was more pronounced for the group of naives during the first $^1/_2$ h, while lactic acid concentration levelled at a minimum value for both groups and remained constant for an additional $2^1/_2$ h.

The effect of THC, in concentrations up to $3.8 \times 10^{-7}$ *M* and of crude hashish up to 100 mg per assay mixture, on LDH activity, was studied as described. The results indicated that there is no effect of these substances on the enzyme activity under the present experimental conditions. The effect of hashish smoking showing the glucose concentration in the blood is stated in the Table. The data clearly demonstrate that, for both groups, glucose remained practically constant throughout the time of the experiment.

*Discussion.* The study of the concentration of various blood constituents can be used as an indication of the action of hormones in the periphery and, therefore, of their mobilization by various drugs.

The present studies have shown that blood glucose, which is affected by the adrenaline of the periphery, does not change after hashish smoking. At first glance this finding would indicate that hashish smoking does not increase blood adrenaline levels. However, alternative effects of hashish, which would inhibit the action of adrenaline with respect to carbohydrate metabolism, cannot be excluded.

A prominent finding of the present experiments is the decrease of blood lactic acid immediately after hashish smoking. The rate of decrease was higher in the group of naives as compared with chronic smokers It is known that marihuana smoking by subjects without previous experience causes an increase in peripheral blood flow[12]. On this basis, the fall of lactic acid after hashish smoking can be explained by this circulatory adjustment which may, possibly, lead to decreased production in the well-oxygenated muscle. The earlier fall of blood lactic in the group of naives, as compared to chronic smokers, suggests that mechanisms involved in the increase of peripheral blood flow rate and in the production of lactic acid, react more quickly when hashish is smoked by subjects without previous experience.

*Résumé.* Détermination de glucose et d'acide lactique contenus dans le sang de fumeurs chroniques et volontaires inexpérimentés à différents intervalles. Le niveau du glucose n'a pas été changé et il est donc probable qu'à la périphérie les catécholamines ne sont pas affectées. L'action possible du hashish sur la déshydrogénase lactique active a été testée in vitro avec de l'enzyme pure et aucun effet n'a été relevé.

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*21 January 1974.*

Effect of hashish smoking on the glucose concentration in the blood

| Time (min) | Glucose ± SD (mg/100 ml) Naives | Chronic |
|---|---|---|
| 0[a] | 114 ± 18 | 100 ± 10 |
| 30 | 109 ± 13 | 102 ± 2 |
| 60 | 105 ± 14 | 104 ± 5 |
| 90 | 106 ± 13 | 102 ± 3 |
| 120 | 105 ± 12 | 103 ± 7 |
| 150 | 106 ± 8 | 103 ± 4 |
| 210 | 102 ± 7 | 105 ± 6 |

[a] Before smoking. Data are mean values ± SD for 5 subjects in each group in the indicated time intervals.

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[13] Acknowledgment. The authors are gratefull to Bio-Check International S.A. for the permission of using Technicon Auto-analyzer for the determination of glucose.