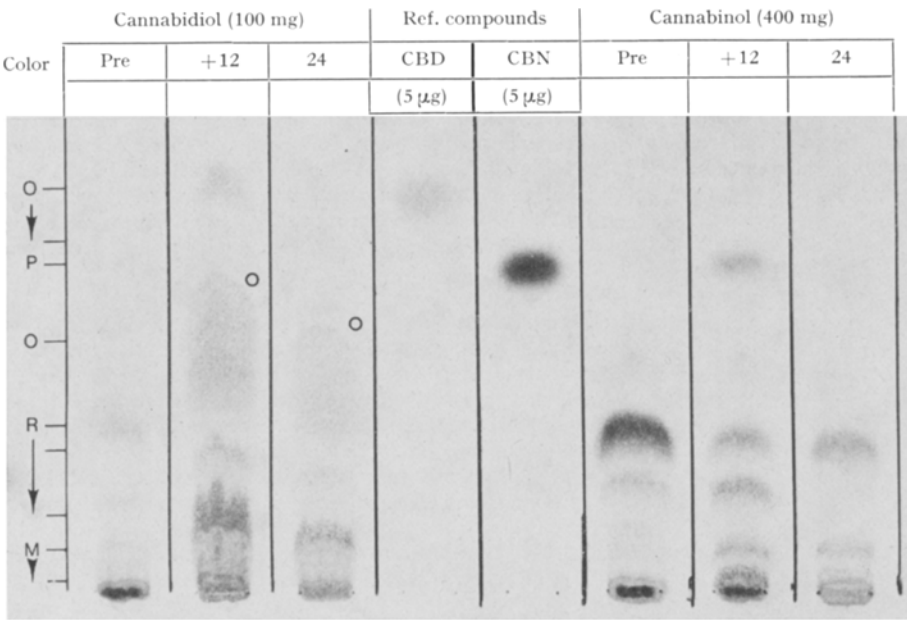

Fig. 2. Thin-layer plate of urine fractions prior to treatment and during the first and second 12-h periods after oral doses of 100 mg of CBD and 400 mg of CBN. Note presence of unchanged CBD and CBN during the first 12 h as well as appearance of new spots of a slower-moving, more polar type. Abbreviations: O, orange; P, purple; R, red; M, magenta coloring of spots.

active doses of both THC isomers and their metabolites to man. Because CBN is less compatible than CBD with any aqueous medium, this route of administration was not used for this substance.

Results. Oral doses of CBD and CBN started with 10 mg, a dose of THC usually showing definite, though moderate clinical effects in man. At no oral dose level were any of the characteristic mental or physical effects of THC observed. Urine samples taken from the subjects receiving the maximum oral doses were subjected to thin-layer chromatography, using techniques previously described[7]. Both CBD and CBN, unlike THC, were excreted in part unchanged. (Figure 2) Other metabolites related to their intake were also observed as new spots on the chromatograms. The initial i.v. injection of CBD was 5 mg; an equal dose of THC produces a strong and lasting effect by this route. As no reaction was noted, larger doses were subsequently used (10, 20 and 30 mg). None of the characteristic mental effects of cannabis were observed, as well as a complete absence of any change in pulse rate or degree of conjunctival reddening.

Discussion. The inactivity of these cannabinoids has been confirmed in man. In part, this difference from THC may be due to a somewhat different metabolism of CBD and CBN. It is still possible that these cannabinoids, although inactive themselves, might interact with THC to alter its effects. CBN pretreatment antagonized the prolongation of sodium pentobarbital sleeping time produced by THC in mice[8]. CBD inhibited not only the metabolism of THC but also that of tis primary metabolite, 11-hydroxy-THC, a compound with at least the same degree of activity as THC itself. Thus, CBD might enhance the effects of THC without having direct THC-like actions. As the assertion is often made, and widely believed, that different types of cannabis have different patterns of pharmacological effects independent of differences in THC content, such interactions might still afford an explanation for such differences. These differences cannot be attributed to direct pharmacological effects of CBD or CBN themselves.

Résumé. Le cannabidiol administré à dose de 100 mg par os et de 30 mg par injection i.v. fut inactif dans les sujets d'étude. Le cannabinol, à dose orale de 400 mg, le fut aussi. Ces constituants du cannabis ne contribuent donc pas à l'effet pharmacologique.

L. E. HOLLISTER

Veterans Administration Hospital Palo Alto (California 94304, USA), 4 January 1973.

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The Effect of Pentobarbital on Brain 5-HT Metabolism in Mice

BONNYCASTLE, GIARMAN and PAASONEN[1] observed an increase of brain serotonin (5-HT) concentration after i.p. injection of pentobarbital in rats. It seemed of interest to us to investigate whether this increase was due to an accelerated synthesis or a decreased metabolism of 5-HT.

Materials and methods. Male NMRI/Han mice of 25–35 g were kept in groups of 10 at room temperature (20–22 °C) on an ad libitum diet of food and water. The mice were killed by decapitation. The brains were kept in 96% ethanol for 30–60 min. This treatment was without influence upon brain serotonin concentration. The neo-

[1] D. D. BONNYCASTLE, N. J. GIARMAN and M. K. PAASONEN, Br. J. Pharmac. Chemother. *12*, 228 (1957).

cortex and the cerebellum were then removed and discarded and the remaining parts were frozen in liquid nitrogen and stored at −20 °C until serotonin estimation. For that purpose, brains of three animals were pooled, weighed and homogenized in 0.4 *N* perchloric acid for 60 sec with a polytron ultrasonic homogenizer. The precipitated proteins were removed by centrifugation and 1 ml of the supernatant was mixed with 1 g NaCl, 3 ml borate buffer (pH 12.2), 0,27 ml 1 *N* NaOH and shaken with 5 ml heptanol for 10 min. Then 4.5 ml of the heptanol phase were mixed with 0.7 ml 0.1 *N* HCl and 10 ml n-heptane and shaken for 15 min. In the aqueous phase, the total serotonin was estimated by fluorimetry[2]. The ^{14}C-serotonin, which was formed after i.p. injection of 1 μCi/10 g body weight of DL-tryptophan-methylene-^{14}C, was determined in the same aqueous extracts, which were used also for total 5-HT estimation, with the help of a dioxane scintillation fluid. The total ^{14}C-content in brain was estimated by dissolving 0.2 ml of the homogenate in 3 ml soluene (Packard) and counting in 10 ml of a dioxane scintillation fluid, using a Beckman LS-250 Liquid Scintillation Counter.

Increase of 5-HT concentration in mouse brains after pargyline injection

Time after pargyline injection (min)	5-HT concentration (ng/g wet tissue) Mean ± S.D.	
	Controls	Pentobarbital-treated
45	690 ± 19	680 ± 19
75	741 ± 15	736 ± 21

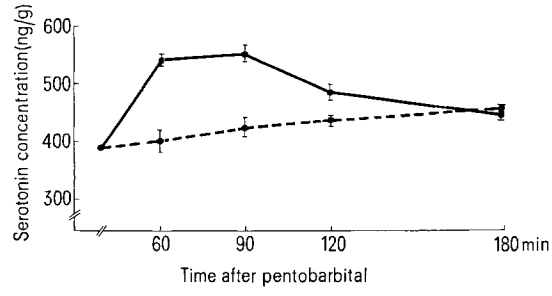

Fig. 1. Brain serotonin concentration. Continuous line, pentobarbital-Na-treated mice (50 mg/kg i.p.); dotted line, saline controls; abscissa, min after pentobarbital injection; ordinate, serotonin concentration in brain (ng/g wet tissue). Mean of 6 values ± S.D.

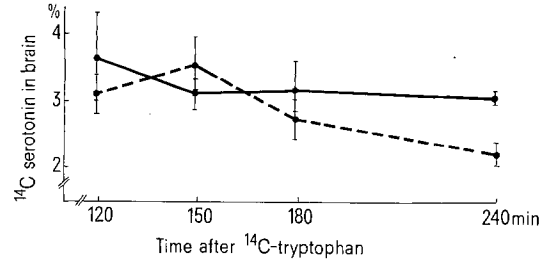

Fig. 2. ^{14}C-serotonin percentage in total brain radioactivity. Continuous line, pentobarbital-NA-treated mice (50 mg/kg i.p.); dotted line, saline controls; abscissa, min after ^{14}C-tryptophan injection; ordinate, ^{14}C-serotonin/total ^{14}C in brain × 100%. Mean of 6 values ± S.D.

Results and discussion. As shown in Figure 1, 60 and 90 min after i.p. injection of 50 mg/kg pentobarbital-Na, the total serotonin concentration was significantly elevated, while after 180 min it had returned to control values. This pentobarbital dose induced only a slight anaesthesia at stage I or II, lasting for about 90–120 min.

To study the serotonin synthesis in the presence of pentobarbital, mice were injected with the MAO inhibitor pargyline (75 mg/kg i.p.) and 15 min later with pentobarbital-Na (50 mg/kg i.p.). The inhibitor led to an elevation of the serotonin concentration in the brains 45 and 75 min after its injection (30 and 60 min after pentobarbital), but there was no difference between pentobarbital-treated mice and controls (Table). These results suggest that pentobarbital is without influence upon the serotonin biosynthesis in brains of mice.

The ^{14}C-serotonin percentage of the total ^{14}C content in brains of mice treated with pentobarbital 60 min after ^{14}C-tryptophan injection is shown in Figure 2. 120, 150, and 180 min after ^{14}C-tryptophan injection, no statistically significant difference between pentobarbital-treated mice and controls could be determined, while 240 min after ^{14}C-tryptophan injection (180 min after pentobarbital) the percentage was much higher in pentobarbital-treated mice than in controls ($p < 0.001$, Student's *t*-test).

These results suggest that the removal of serotonin from the brains is decreased by pentobarbital. In principle, it is also possible that the relative slowing down of the ^{14}C-serotonin decrease after pentobarbital could be simulated by an increased synthesis of proteins, which are the main pool of ^{14}C in brain under our experimental conditions. However, previous results excluded that barbiturates increase the turnover of the proteins. On the contrary, their turnover is even decreased in some subcellular fractions[3].

It is concluded that pentobarbital decreases the serotonin turnover, while the synthesis is not affected. This possibly explains the elevation of the serotonin concentration in the brain stem, which was not observed in cortical tissue (unpublished observations). The present results are in agreement with those of CORRODI, FUXE and HÖKFELT[4], who observed that pentobarbital inhibited the depletion of rat brain serotonin after inhibition of serotonin synthesis by α-propyldopacetamide.

Zusammenfassung. Bei Mäusen erhöhte Pentobarbital den Serotoningehalt des Gehirns, ein Effekt, der sich durch eine Verminderung der Umsatzgeschwindigkeit bei unveränderter Synthese des Serotonins erklären lässt.

K. KUSCHINSKY, G. SEIDEL, E. REETZ and C. MEYER-BURGDORF[5]

Abteilung Biochemische Parmakologie, Max-Planck-Institut für experimentelle Medizin, Hermann-Rein-Strasse 3, D-3400 Göttingen (Germany) 3 January 1973.

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[5] Acknowledgment: We are indebted to the Deutsche Abbott GmbH for a generous gift of pargyline.