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Rabbit reactivity to cannabis preparations, pyrahexyl and tetrahydrocannabinol

SIR,—Recent work on the chemistry of cannabis constituents and synthesis of tetrahydrocannabinols (Gaoni & Mechoulam, 1965; Taylor, Lenard & Shvo, 1966) will certainly renew an interest in their pharmacological activities, relative potency and stability. Among the biological tests so far proposed for cannabis and related principles, the abolition of the rabbit blink reflex appears to be one of the more sensitive (Gayer, 1928). We believe this test to be suitable for the estimation of one of the central actions of marihuana.

The assay is made in groups of 3–6 rabbits repeatedly injected with a preparation until the blink reflex is completely abolished. In preliminary tests a rough estimate of the potency is made and less reactive animals are discarded. The selected rabbits are restrained in wooden cages, with the head out, and maintained in a noiseless room. The test solution is made in saline with polysorbate 80 and the emulsion slowly injected into the ear vein. Twenty stimuli in each eye are made with a horse hair at 10 min intervals and the number of injections (0·2 ml/kg each) to completely abolish the blink reflex in both eyes is determined. For the estimation of the relative potency of the unknown preparation in terms of a standard, two groups of at least 6 animals each should be employed.

The dried and powdered flowering tops and leaves of the plant cultivated in north-east Brazil or in the neighbourhood of these laboratories were extracted with light petroleum for 4–6 hr. The extract was filtered through activated charcoal, washed with water and evaporated to dryness. The residue was dissolved in acetone and kept in the refrigerator overnight to separate wax constituents. After acetone evaporation the crude resin was dissolved in light petroleum and chromatographed on an alumina column. Elution with light petroleum, light petroleum with benzene, benzene, and benzene with sulphuric ether afforded fractions which were examined in a Beckman DU spectrophotometer at 250 and 280 mμ. Details and properties of the main components are given elsewhere (Valle & Hyppolito, 1964).

The most active fractions obtained after chromatography on alumina columns were those designated Cr19Fr10 and Cr23Fr14. Besides these cannabis preparations, synthetic samples of pyrahexyl and tetrahydrocannabinol (THC) were also assayed.

A sample of cannabis crude resin (0·8 mg/ml) was assayed against THC (0·08 mg/ml) as standard. The results (mg/kg $\pm$ s.d.) were 0·107 $\pm$ 0·013 (6 animals) and 1·143 $\pm$ 0·335 (6 animals) respectively and indicated a relative potency of 0·093 and that the solutions tested did not differ significantly in their activities ($F = 0{\cdot}105$, $P < 0{\cdot}05$). Then, by our procedure, the selected cannabis

resin exhibited about 10 times less activity, on a weight basis, than the sample of synthetic THC, adopted as a reference compound. We have also assayed our fractions Cr19Fr10 and Cr23F14, obtained strictly in the same way from the same batch of starting drug material. Solutions of both preparations, containing 20 μg/ml each, showed similar potencies: 0·031 $\pm$ 0·011 and 0·034 $\pm$ 0·010 mg/kg.

TABLE 1. REPEATED ASSAYS OF A CANNABIS CRUDE RESIN AND OF TETRAHYDROCANNABINOL

Date	No. of animals	Mean and limits of body weight (kg)	Test solution	Activity mg/kg (Mean and limits)	F‡
May 25, 1965	4	2·6 (2·0–3·6)	Can.* (2 mg/ml)	3·19 (2·32–3·50)	
Jan. 28, 1966	4	3·1 (2·7–3·5)	,, ,,	2·99 (2·40–3·60)	0·29
Nov. 9, 1965	3	2·8 (2·6–3·0)	THC† (0·1 mg/ml)	0·16 (0·12–0·19)	
Nov. 17, 1965	4	3·0 (2·6–3·5)	,, ,,	0·09 (0·09–0·12)	
Dec. 10, 1965	6	2·8 (2·5–3·6)	,, (0·08 mg/kg)	0·11 (0·08–0·19)	
Jan. 28, 1966	5	2·7 (2·3–2·9)	,, (0·1 mg/kg)	0·10 (0·06–0·13)	2·16

* Cannabis crude resin dated April 1962; ethanolic solution (10 mg/ml) kept at 4°. Dilution with saline plus polysorbate 80 before using. Animals intravenously injected (0·2 ml/kg) every 10 min until the blink reflex is completely abolished.

† Stock solution in ethanol (10 mg/ml) kept for 5 months in dark glass container at room temperature

‡ Values of F at a probability level of 0·05, not significant.

The sample of pyrahexyl was active at 0·68 $\pm$ 0·29 mg/kg rabbit weight. The results of repeated assays of a cannabis crude resin and of THC are given in Table 1 from which the stability of the preparations used may be deduced.

In conclusion, rabbit reactivity to cannabis, tetrahydrocannabinol and pyrahexyl showed that the ethanolic solutions of these agents maintained their activities for months.

Acknowledgements. We wish to thank Rockefeller Foundation (RF-58217) and the "Fundação de Amparo à Pesquisa do Estado de São Paulo" (Proc. 71/62 & 63/337) for their financial support.

A ten year old sample of Pyrahexyl was kindly supplied by Dr. R. K. Richards, Research Division, Abbott Laboratories, North Chicago, Ill. Tetrahydrocannabinol was obtained through the courtesy of Dr. Milton Joffe and Dr. Francis Morthland, U.S. Defense Office (AROLA).

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