a dinner in honor of Dr. Bear at 6 o'clock on the same evening. An hour of conversation following the dinner will be led by Dr. Bear. The topic will be "Soils, Plants, Animals, Man." Dr. Bear was formerly chairman of the department of soils at the Ohio State University.

SPECIAL ARTICLES

MARIHUANA ACTIVITY OF CANNABINOL[1]

IT is generally agreed that cannabinol, the aromatic analog of the hydro-aromatic active principles of hemp oil, is entirely inactive[2] and certainly not responsible for the characteristic pharmacological activity of hemp.[3,4,5,2,6] However, conclusions are valid only within the limits of the underlying test. Bioassay of hemp components and related compounds has been limited by their scarcity and by the toxicity of the solvents necessary to obtain solution. In our earlier experiments[7] done for the purpose of comparing synthetic compounds with the highly potent natural drug principles, there was little interest in a substance when its assay indicated a potency of less than 1/200 that of charas tetrahydrocannabinol. The availability of larger quantities of cannabinol and the finding that propylene glycol is an effective solvent for intravenous preparations have made possible a study of the effects of larger doses.

Two preparations of cannabinol were employed, one (I) a repeatedly recrystallized batch prepared from the outer crust of aged charas[8] (M.P. 75.0–75.5° C),[9] the other (II) a crude synthetic cannabinol.[10,11] The amount available was sufficient for six experiments in dogs. The substances were administered intravenously in concentrated solution in propylene glycol. The results are shown in Table 1. All doses over 12.2 mg/kg produced marked ataxia. The effects are graded from I to IV in accordance with the intensity of effect as described in detail elsewhere.[7] The action was in all respects identical with that of the natural tetrahydrocannabinols. The duration of ataxia varied from two to more than 24 hours according to the dose. The results indicate a potency of about 0.04.[12] The potency of the pure substance (I) may be somewhat higher.

TABLE 1

ATAXIA ACTIVITY OF CANNABINOL IN THE DOG BY INTRAVENOUS ADMINISTRATION IN PROPYLENE GLYCOL

Sample of cannabinol	Weight of animal kg	Cannabinol		Grade of ataxia
		Dose mg/kg	Concentration gm/100 cc	
I	8.98	12.2	62	I–II
I	7.95	18.1	20	III–
II	6.56	32.5	20	IV–
II	7.80	38.5	20	III–IV
I	6.84	52.0	62	III–
I	6.60	254.0	62	III–IV

In view of the high purity of the one and the synthetic procedure by which the other was prepared, the ataxia activity of these specimens must evidently be ascribed to the cannabinol molecule rather than to an impurity. Therefore cannabinol must be included among the compounds having marihuana activity. This signifies that the end-product of the process of oxidative degradation of tetrahydrocannabinol[9] from which the cannabinol of hemp resin results, is not entirely devoid of marihuana activity. The comparatively low potency of cannabinol is of little practical significance, and even the large amounts contained in some hemp oils contribute little to the total activity because of the presence of highly potent tetrahydrocannabinols. However, the activity of cannabinol is of interest from the aspect of the relationship between structure and activity, providing one more example of the fact that under natural conditions there is not necessarily a fundamental difference between aromatic and hydro-aromatic compounds. This agrees with our prior experience that the variations in spatial arrangement due to the presence and position of a double bond in ring A, while having considerable quantitative influence upon the potency, do not determine the presence or absence of marihuana activity.[7] The activity is neither absent when, as shown previously, an aliphatic cyclohexane[13] or -heptane[7] or even a pair of open alkyl chains,[14] nor, as this study shows, when an aromatic ring takes the place of the hydroaromatic ring of the natural or synthetic marihuana-active dibenzopyran derivatives.

[1] Aided by a grant from Abbott Laboratories, Chicago.

[2] A. D. Macdonald, *Nature*, 147: 167, 1941.

[3] F. Bergel and A. R. Todd, *Biochem. Jour.*, 33: 123, 1939.

[4] A. H. Blatt, *Jour. Washington Acad. of Sci.*, 28: 465, 1938.

[5] R. S. Cahn, *Jour. Chem. Soc.*, 1933: 1400.

[6] H. Marx and G. Eckhardt, *Arch. Exp. Pharm.*, 170: 395, 1933.

[7] S. Loewe, in: "The Marihuana Problem." Lancaster, The Jaques Cattell Press. 1944.

[8] From the Narcotics Laboratory, Washington, D. C., through the courtesy of Dr. J. Levine.

[9] J. Levine, *Jour. Am. Chem. Soc.*, 66: 1868, 1944.

[10] R. Adams, B. R. Baker and R. B. Wearn, *Jour. Am. Chem. Soc.*, 62: 2204, 1940.

[11] Through the courtesy of Dr. Roger Adams, Department of Chemistry, University of Illinois.

[12] Synthetic racemic (7,8,9,10-)tetrahydrocannabinol having 1/15 the potency of charas tetrahydrocannabinol was used as a standard, as in previous studies. (See reference 7.)

[13] R. Adams, S. Loewe, B. C. Pease, C. K. Cain, R. B. Wearn, R. R. Baker and H. Wolff, *Jour. Am. Chem. Soc.*, 62: 2566, 1940.

[14] R. Adams, C. K. Cain and S. Loewe, *Jour. Am. Chem. Soc.*, 63: 1977, 1941.

Summary: Cannabinol, generally believed to be an inert component of hemp oil, is shown to have marihuana activity. The significance of this observation with regard to the relationship between structure and activity in the class of cannabinols is discussed.

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SOME EFFECTS OF SALTS ON TRUE CHOLINESTERASE

THE observations to be reported here are concerned with the effects which salts in various concentrations exert on the action of true cholinesterase at different levels of acetylcholine. Experiments have been published previously on the effects of salts on cholinesterase; their results, however, could not be fully evaluated at that time because the differentiation had not been made between the specific or true cholinesterase which hydrolyzes certain choline esters, and choline esters only, and the non-specific or pseudo-cholinesterase which is capable of hydrolyzing not only some esters of choline but a variety of non-choline esters as well.[1] The earlier discovery by Alles and Hawes[2] of differences between the cholinesterases in human erythrocytes and human serum, interesting as such and fully recognized by us,[1] did not—as recently suggested by Glick[3]—touch upon the criterion, specificity towards choline esters or non-specificity, without which no differentiation could have been made between the cholinesterases throughout the animal kingdom[4] and no basis provided for the experiments to be presented here.

Glick[5] found that the cholinesterase activity of rabbit serum, but not that of horse serum, towards acetylcholine in a concentration of 375 mg per cent. was increased by sodium and potassium chloride. We now know that rabbit serum contains predominantly true cholinesterase, and it will become clear in the course of this paper that the increase in enzymatic activity in the presence of salts was due to the increased activity of true cholinesterase.

Alles and Hawes[2] found that the cholinesterase activity of human erythrocytes, now known to contain true cholinesterase only,[1] was greatly potentiated when the sodium chloride concentration was increased. Furthermore, the curves they present demonstrate a shift in the optimum substrate concentration from 0.00025 M (4 mg per cent.) acetylcholine in the presence of 0.034 per cent. NaCl to 0.00075 M (12 mg per cent.) acetylcholine in the presence of 0.85 per cent. NaCl, though the authors make no mention of this displacement in the analysis of their results.

Recently, Nachmansohn and Rothenberg[6] reported that the cholinesterases from erythrocytes and mammalian brain, previously shown to contain true cholinesterase only,[7] display their maximum activity at acetylcholine concentrations of 0.0057 M (90 mg per cent.) and 0.01 M (160 mg per cent.) respectively—concentrations which are very much higher than those reported by Alles and Hawes and by ourselves in a former publication.[1] However, Nachmansohn employs a very high salt concentration in his medium (0.215 M.), and though at first sight his findings seem to contradict our results, actually they support them, as will be shown later in this communication.

In our experiments we determined the activity-substrate concentration curves of true cholinesterase from different sources in a medium containing 0.025 M sodium bicarbonate and also in media in which, in addition to 0.025 M sodium bicarbonate, salts in varying concentrations were present.

The enzyme activity was measured manometrically at 37.5° C. by Warburg's method, the enzyme solution being placed in the main compartment of the Warburg flask and acetylcholine in the sidearm; the total volume of the fluid, which was saturated with 5 per cent. CO_2, was 6.0 ml. A control vessel was prepared for each concentration of acetylcholine, in order to correct for spontaneous hydrolysis. The acetylcholine in the control and experimental vessels was tipped into the main compartments of the respective vessels simultaneously and readings were taken immediately after re-establishment of temperature equilibrium. In all cases the activity was calculated for a twelve-minute reaction period. Since at concentrations of acetylcholine lower than 0.001 M (16 mg per cent.) the reaction time is limited by the small amount of substrate available, it was necessary in such instances to take readings at one-minute intervals. The enzyme activity at the 0.000125 M (2 mg per cent.) acetylcholine level was obtained from the rate of hydrolysis observed after calculations had shown that one half of the original acetylcholine present in a 0.00025 M (4 mg per cent.) solution had been hydrolyzed. The figures recorded at the 0.000125 M level can therefore be considered as an approximation only.

In the sera and parotid glands of ox and sheep, in mammalian erythrocytes and in the brain tissue of any vertebrate, the specific or true cholinesterase alone is responsible for the hydrolysis of acetylcholine. The enzyme from the above sources, when measured in a medium containing no salts other than 0.025 M sodium bicarbonate, is subject to excess substrate inhibition and displays its maximum activity at low acetylcholine

[1] B. Mendel and H. Rudney, *Biochem. Jour.*, 37: 59, 1943.
[2] G. A. Alles and R. C. Hawes, *Jour. Biol. Chem.*, 133: 375, 1940.
[3] D. Glick, SCIENCE, 102: 100, 1945.
[4] B. Mendel and H. Rudney, SCIENCE, 100: 499, 1944.
[5] D. Glick, *Nature*, 148: 662, 1941.
[6] D. Nachmansohn and M. A. Rothenberg, *Jour. Biol. Chem.*, 158: 653, 1945.
[7] B. Mendel and H. Rudney, SCIENCE, 98: 210, 1943.