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.

concentrations ranging from about 0.00015 M to 0.0005 M (2.5–8.0 mg per cent.).[1]

Fig. 1 shows the activity-substrate concentration curves obtained with mouse brain. In the absence of any salts other than 0.025 M sodium bicarbonate

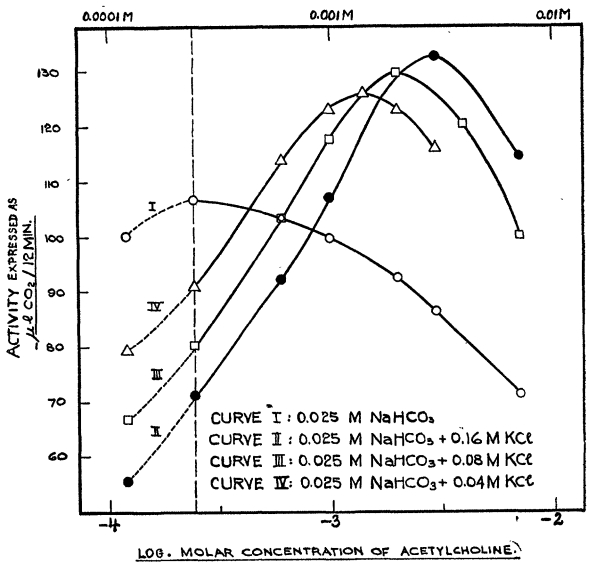

FIG. 1. Hydrolysis of acetylcholine by true cholinesterase as a function of acetylcholine concentration. The broken vertical line marks the 0.00025M (4 mg per cent.) acetylcholine level. The cholinesterase was obtained from mouse brain; 1 g of the tissue was ground and suspended in 5 ml water and 0.2 ml of this suspension was used in all experiments.

(Curve I) the optimum activity is displayed at 0.00025 M (4 mg per cent.) acetylcholine. When potassium chloride in a concentration of 0.16 M is added to the above medium (Curve II) we not only find a shifting of the region of maximum activity from 0.00025 M (4 mg per cent.) to about 0.003 M (49 mg per cent.), but also a pronounced reduction of the enzyme activity at its former optimum of 0.00025 M acetylcholine. Similar effects are obtained with lower concentrations of potassium chloride. In the presence of 0.08 M potassium chloride (Curve III) the optimum substrate concentration is 0.002 M (32 mg per cent.) acetylcholine and the activity at 0.00025 M acetylcholine, though reduced in comparison with the activity found in the presence of 0.025 M sodium bicarbonate alone, is greater than that obtained in the presence of 0.16 M potassium chloride; in the presence of 0.04 M potassium chloride (Curve IV) the enzyme displays its optimum activity at 0.0015 M (24 mg per cent.) acetylcholine, and the decrease in activity at 0.00025 M acetylcholine is still less marked than it is in the presence of the higher salt concentration.

Our experiments show that the relationship between enzyme activity and substrate concentration is changed by the addition of salt to the medium. A stepwise increase in the salt concentration, though not abolishing the excess substrate inhibition as such, causes a gradual shifting of the optimal activity of the enzyme to higher levels of acetylcholine—a fact which brings to light the cause of the discrepancy between Nachmansohn and Rothenberg's results and our own. Furthermore, with increasing concentrations of potassium chloride an absolute increase in the rate of acetylcholine hydrolysis occurs at the new optimum levels and beyond these points as well. Similar results are obtained with true cholinesterase from other sources mentioned previously in this paper.

When sodium chloride instead of potassium chloride is added to the medium in equimolar concentrations, the activity-substrate concentration curves show the same general trend but they are not identical. This fact reveals the possibility of superimposed specific effects of particular ions which will have to be studied later in detail.

The above observations point to the important role ions play in the hydrolysis of acetylcholine by true cholinesterase. The escape of potassium from cells, occurring upon stimulation,[8] might possibly help to maintain optimal conditions for the cholinesterase activity which otherwise, *i.e.*, if the potassium level in the cell remained constant, would drop sharply with declining concentrations of acetylcholine. Conversely, an increase of potassium in the extracellular fluid, shown by various workers[9,10,11] to sensitize cells to submaximal doses of acetylcholine, could cause this effect by retarding the escape of potassium from these cells, thereby creating conditions in which the activity of true cholinesterase towards low concentrations of acetylcholine would be suboptimal.

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THE EFFECT OF CORN STEEP LIQUOR ASH ON PENICILLIN PRODUCTION[1]

IT generally is assumed that corn steep liquor enhances penicillin production because of a specific organic constituent, but during a study of the physiology of *Penicillium chrysogenum* it was found that the inorganic constituents of corn steep liquor played an important role, as will be shown in the following results from preliminary studies.

[8] M. Vogt, *Jour. Physiol.*, 86: 258, 1936.
[9] W. Feldberg and A. Vartiainen, *Jour. Physiol.*, 83: 103, 1935.
[10] G. L. Brown and W. Feldberg, *Jour. Physiol.*, 86: 290, 1936.
[11] D. W. Bronk, *Jour. Neurophysiol.*, 2: 380, 1939.

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.