16721

Effect of Diisopropyl Fluorophosphate (DFP) on the QO_2 of the Rabbit Cerebral Cortex.

SYDNEY ELLIS.* (Introduced by G. S. Eadie.)

From the Biochemistry Section, Medical Division, Army Chemical Center, Md.

Several reports indicate that diisopropyl fluorophosphate (DFP), or related compounds, may be of clinical value in view of their prolonged inhibition of cholinesterase activity. When injected into humans, stimulation of cholinergically innervated tissues has been noted. The central nervous system also appears to be affected.[1,2] Since 'central' effects have not been noted when neostigmine is administered by the usual routes,[3] or in large doses by continuous infusion,[4] the suggestion has been made that this difference in the action of the two agents is due to the greater fat solubility of DFP in contrast to neostigmine.[1,2]

Another possibility exists, *i.e.*, that DFP, in addition to inhibiting cholinesterase, might affect the metabolism of the brain. This has been investigated in rabbits injected with DFP in doses sufficient to inactivate cholinesterase.

Methods. Normal rabbits were sacrificed as controls by injecting air intravenously. Diisopropyl fluorophosphate at a concentration of 1:1000 freshly dissolved in physiological saline was injected intravenously in amounts stated in the table. The animals were used immediately following death or, if not dead 30 minutes after the injection of DFP, they were sacrificed by injecting air intravenously. Immediately after death the cranium was opened and the cerebrum removed intact.

Cerebral cortex slices were removed from one cerebral hemisphere by the tissue slice technic of Deutsch.[5] The remaining hemisphere was homogenized in 0.025 M sodium bicarbonate solution[6] with the Potter all-glass homogenizer and made up to a final dilution of 1:10 with the bicarbonate solution.

Cholinesterase activity was determined by Ammon's method[7] with 0.5 cc of the 1:10 brain homogenate in a final concentration of 0.015M acetylcholine bromide in 0.025M sodium bicarbonate at 38°C. The gas phase was 5% CO_2 and 95% N_2.

The oxygen uptake, expressed as cu mm oxygen utilized per mg of dry weight of tissue per hour (Q_{O_2}), was determined on cortical slices suspended in 3 cc of a salt mixture of the following composition[8]: 124.6 mM sodium chloride; 4.01 mM potassium chloride; 1.04 mM calcium chloride; 0.52 mM magnesium chloride; 10 mM phosphate buffer, pH 7.4; 0.20 g% anhydrous glucose. The dry weights of the tissues were obtained after the tissues were heated at 105°C for at least 16 hours.

The data of Table I demonstrate that DFP does not change the oxygen uptake of cerebral cortex slices of rabbits treated with this compound, as compared with untreated rabbits, even when acetylcholine hydrolysis by the cerebrum is completely inhibited. This does not eliminate the possibility of a more subtle

* Formerly Captain, Sanitary Corps, A.U.S. Present address: Department of Physiology and Pharmacology, Duke University School of Medicine, Durham, N. C.

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TABLE I.
Oxygen Uptake and Cholinesterase Activity of Brain of Normal and DFP-Treated Rabbits.

DFP mg/kg i.v.		Cholinesterase activity cu mm CO/30 min/ 0.5 cc[2] homogenate	Q_{O_2}
0.0		233	11.2
0.0		299	10.9
0.0		246	9.6
0.0		222	10.2
0.0		244	9.9
0.0		233	10.3
0.0		239	10.5
0.0		221	9.9
	Avg	242	10.3
0.5		4	9.9
0.5		4	10.2
0.5		4	10.6
1.0		4	8.8
1.0		—0.7	10.4
1.0		—1.1	11.4
	Avg	2	10.2

All values are averages of duplicate or triplicate determinations.

effect on brain metabolism. It does show, however, that DFP has no appreciable effect on the normal oxygen uptake of cerebral cortical slices, and that cholinesterase activity is not essential for such oxygen uptake.

Discussion. Some related studies of the effect of DFP on enzymes other than esterases have been reported. Webb[9] found that the following enzyme systems were not affected by DFP at concentrations of M/500 to M/2000: cytochrome oxidase, succinic dehydrogenase, hexokinase, Robison ester glycolysis by muscle powder, choline, dehydrogenase, catalase, carboxylase, ptyalin, peroxidase, lactic dehydrogenase of muscle, diaphorase, liver alcohol dehydrogenase, and carbonic anhydrase. Hunt[10] has stated that the normal oxygen uptake by liver homogenate and the additional oxygen uptake due to added ethyl butyrate are not inhibited noticeably by DFP.

The increased respiration of rat brain slices observed by Levy[11] when the tissue was in concentrations of eserine salicylate between 1:500 and 1:1000 are attributable to the enormous concentration of the drug. It may be that the salicylate ion is being metabolised at a sufficient rate at this high concentration to produce the increased oxygen uptake attributed to eserine.

Summary. When injected into rabbits in lethal doses, diisopropyl fluorophosphate had no appreciable effect on the oxygen uptake of cerebral cortex slices of these animals even though cholinesterase was completely inhibited.

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16722 P

In vitro Studies of a Growth Inhibitory Fraction Obtained from Adult Sheep Cardiac Muscle.*

R. S. HOFFMAN, JAMES A. DINGWALL AND WILLIAM DEW. ANDRUS.
(with the technical assistance of Irene Jacob.)

From the Department of Surgery, New York Hospital and Cornell University Medical College, New York City.

In earlier papers[1,2] we have reported on the growth stimulating effects of a Tyrode's solution extract of animal tissue as demonstrated both *in vitro* and when applied topically to wounds in dog and man. In the course of attempts to fractionate adult animal tissue extracts in order to determine the factors responsible for this stimulation, one crude fraction has been of particular interest because it

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