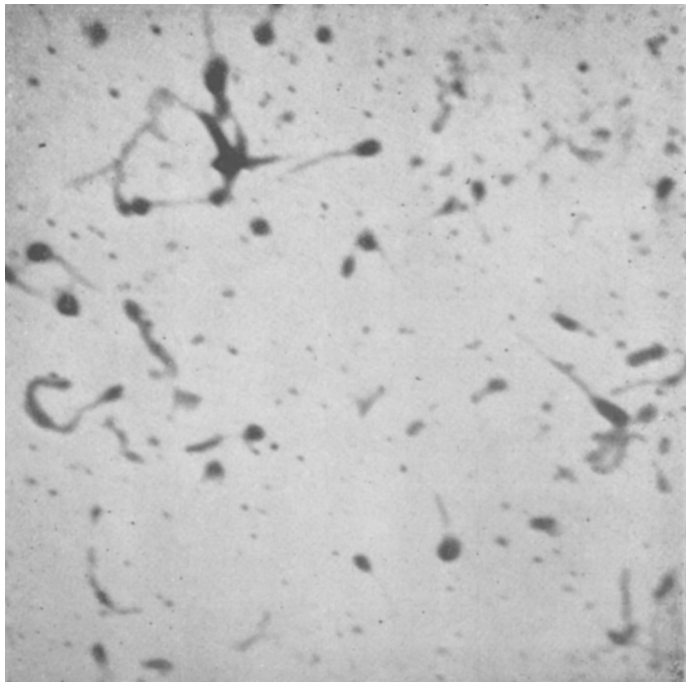

FIG. 2.
Electronmicrograph of same preparation as 1b in .15 M NaCl.
× 17,200.

to the filamentous form previously described. This conversion is prevented by partial inactivation with formaldehyde, mustard gas, or gentle heating.

15725

Effect of Hexaethyl Tetraphosphate on Choline Esterase *in vitro* and *in vivo*.*

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Hexaethyl tetraphosphate, $C_{12}H_{30}P_4O_{13}$, (HTP), has recently been introduced in this country as an insecticide. It was first used in this capacity by the Germans and was uncovered by technical teams following the close of the European phase of the war. The German scientists who were interrogated stated that it had a nicotine-like action and was used for the control of aphids as a substitute for nicotine. The authors of the present report have been unable to find any reference to its mechanism of action other than its nicotinic effects.

The present study was initiated following observation during routine testing[1] that animals showed symptoms similar to those pro-

* Carried out under a contract with Medical Division, Chemical Corps, U. S. Army.

1 Botkin, A. L., Lipton, M. A., and Mangun, G. H., unpublished data.

2 Potter, V. R., and Elvehjem, C. A., *J. Biol. Chem.*, 1936, **114**, 495.

duced by diisopropyl fluorophosphate. Muscular twitching, tonic and tonic-clonic convulsions, involuntary defecation, micturition and salivation were observed. The parallelism was further confirmed by the production of miosis by instillation of a dilute solution in the eyes of rabbits. Maximal miosis occurred in about 5 minutes with one drop of solution containing 4 mg/ml of HTP. Pupillary diameter returned to normal in 5-12 hours in contrast to a parallel test with a solution of diisopropyl fluorophosphate (2 mg/ml) in which the miotic effect persisted for 3 days.

Experimental. Cholinesterase was measured manometrically employing a test system containing 0.3 ml 0.1 M acetyl choline bromide in a final volume of 3.0 ml of calcium-free Ringer-bicarbonate buffer (0.025 M $NaHCO_3$, 0.15 M NaCl, and 0.04 M $MgCl_2$). This system contained either 50 mg homogenized brain, 100 mg homogenized submaxillary glands, 100 mg washed diluted red cells, or 100 mg serum. For ascertaining the effect of hexaethyl tetraphosphate (HTP) on insect cholinesterase the entire thorax from cockroaches was employed after removal of the chitin and homogenization. After gassing for 5 minutes with 95% nitrogen-5% carbon dioxide and equilibrating for 10 minutes at 38°C the acetyl choline was tipped from the side-arm into the main compartment of the Warburg vessel and readings were taken at 5-minute intervals for 30 minutes.

The effect of hexaethyl tetraphosphate on the cholinesterase of rat tissues and cockroach tissue *in vitro* was measured by the addition of solutions of the inhibitor dissolved in the buffer to the test system. The inhibitor was added immediately after solutions were prepared and incubated with the tissue throughout the gassing and equilibration period (15 minutes) before the addition of acetyl choline. A final concentration of 1×10^{-7} M hexaethyl tetraphosphate produced the following per cent inhibition of cholinesterase: brain 47, submaxillary 53, serum 60, erythrocytes 45, and cockroach tissue 58. Thus, hexaethyl tetraphosphate was an effective inhibitor of cholinesterase

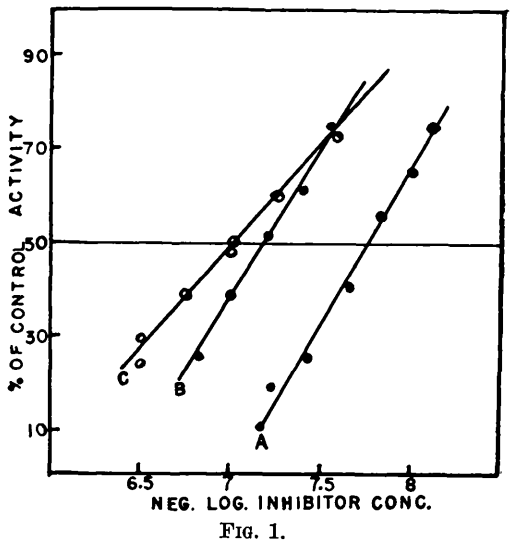

FIG. 1.

The effect of hexaethyl tetraphosphate, diisopropyl fluorophosphate, and a carbamic acid ester on rat brain cholinesterase *in vitro*. Curve A, hexaethyl tetraphosphate; Curve B, di-isopropyl fluorophosphate; Curve C, carbamic acid, N,N-dimethyl-4-dimethylamino-5-isopropyl-phenyl ester, methiodide.

in vitro, all of the tissues showing similar sensitivity toward the compound.

Further evidence of the strong inhibitory action of hexaethyl tetraphosphate on cholinesterase was obtained by comparing its effect on the cholinesterase activity of rat brain *in vitro* with that of diisopropyl fluorophosphate (DFP) and carbamic acid, N,N-dimethyl-4-dimethylamino-5-isopropyl-phenyl ester, methiodide, 2 effective cholinesterase inhibitors. For this comparison a sample of hexaethyl tetraphosphate (Monsanto) and the other compounds were dissolved in the buffer and the measurement carried out as described above. The results shown in Fig. 1 indicate that 50% inhibition of brain cholinesterase activity was obtained by a final concentration of 1.6×10^{-8} M hexaethyl tetraphosphate, 6.3×10^{-8} diisopropyl fluorophosphate, and 1×10^{-7} M carbamic acid ester. Thus, under the conditions of these experiments hexaethyl tetraphosphate was the most effective inhibitor and the similarity in the slopes of the curves in Fig. 1 indicate a similar type of inhibition by DFP and HTP.

Inhibition of cholinesterase *in vivo* was

TABLE I.
Effect of Hexaethyl Tetraphosphate on Cholinesterase Activity of Rat Tissues *in Vivo*.

Dose, mg/kg	% inhibition of activity			Toxicity
	Brain	Submaxillary	Serum	
1	4.5	22	100	0/3 died in 20 minutes
2	45.8	56.5	100	1/3 " " 20 "
3	75.8	83	100	2/3 " " 20 "
5	100	100	100	3/3 " " 5-6 "
10	100	100	100	" " 5 "

demonstrated by administering HTP intraperitoneally to rats and then measuring the cholinesterase activity of the brain, submaxillary glands, and serum by the *in vitro* test system previously described.

The results of these measurements are given in Table I. Values at 1, 2, 3, and 5 mg per kg are the averages of determinations on 3 animals and the 10 mg per kg represents one animal. The cholinesterase values for normal tissues from 10 animals expressed as microliters CO_2 per 50 mg fresh tissue per 10 minutes were: brain 102, submaxillary glands 28, serum 10, and red blood cells 7 and the per cent inhibition in poisoned animals was calculated from these control values.

These *in vivo* experiments demonstrate that HTP inhibits the cholinesterase of all of the tissues examined. Most sensitive was the serum which was completely inhibited by doses of the compound which produce muscular twitching but no lethal effects in the animals. The inhibition of brain and submaxillary gland cholinesterase more nearly paralleled the symptoms and lethal action of the drug with submaxillary gland esterase being inhibited to a somewhat greater extent than brain. Twenty-four hours after the injection of a sublethal dose (1 mg per kg) into rats 35% of the serum activity had returned, at 4 days 70% of the serum activity had returned, and at 8 days the activity had returned to normal.

Summary. Hexaethyl tetraphosphate exerts a strong inhibitory effect on mammalian and insect cholinesterase *in vitro* and *in vivo*. This finding, in conjunction with its gross effects on animals suggests that its physiological effects may be at least in part due to its inhibition of this enzyme.

15726

Antigenic Structure of *Pasteurella pestis* and the Isolation of a Crystalline Antigen.*

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The present report is a summary of investigations initiated in 1942 to extend the knowledge of the antigenic structure of *P. pestis*, and to use this information to place plague prophylaxis on a sounder basis. The detailed experimental data will be reported at a later date.

All the studies, unless otherwise stated, were performed with dried plague bacilli of the virulent "Yreka" strain. The bacilli were grown on agar for 3 days at 37°C and suspended in saline. This was precipitated by one to 2 volumes of acetone cooled to

* The work described in this paper was done under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the University of California.