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.

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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.

—70°C and left overnight. Repeated washings with acetone and drying *in vacuo* produced a bacterial powder of high antigenicity and toxicity (LD_{50} for 20 g mice: 20-40 μg).

Extraction of the acetone-dried plague bacilli with neutral salt solutions (0.85%, 2.5% sodium chloride solutions or 0.4% sodium acetate solution) yielded a water-soluble and a water-insoluble antigenic component. The water-soluble fraction is quite toxic for mice and rats (LD_{50} for 20 g mice, 8-15 μg), and has a high immunogenic value for these species, but a low value for guinea pigs. The water-insoluble portion is nontoxic for mice, and produces a very poor immunogenic response in mice and rats. However, this fraction, when tested as an alum precipitate, has a high immunogenic value, equal to that of whole plague bacilli, for guinea pigs.

The *water-soluble fraction* has been shown to contain at least 3 antigenic components: (1) A carbohydrate protein-soluble at 0.25 saturation of ammonium sulfate at pH 7.0-7.5, and precipitable at 0.3 saturation (Fraction IA). (2) A carbohydrate-free protein soluble at 0.3 saturation of ammonium sulfate at pH 7.0-7.5, and which crystallizes in the form of fine needles when the concentration of ammonium sulfate is raised to 0.33 saturation (Fraction IB). (3) A toxic fraction soluble at 0.33 saturation of ammonium sulfate at pH 7.0-7.5 and almost completely precipitated at 0.55-0.67 saturation (Fraction II). Both Fractions IA and IB are electrophoretically homogenous at pH 8.7 and pH 5.5. They both have approximately the same mobility at both pH values. Fraction IA has the following analytical values: Nitrogen 15.07%, sulfur 0.58%, phosphorus 0.08%, a strongly positive Molisch test, and is highly viscous. Fraction IB has the following analytical values: Nitrogen 15.71%, sulfur 0.59%, phosphorus 0.04%, a negative Molisch test, and a low viscosity. Both fractions are free of agar as determined by serological tests with a sample of horse serum containing antibodies to agar.

Fractions IA and IB are similar immunogenically. They both produce potent antisera in rabbits, and will absorb all of the antibody from sera prepared against either. Sera to these fractions agglutinate plague bacilli to at least the same titer as sera prepared against whole plague bacilli. In one experiment, a serum to Fraction IA had as high a protective value for mice as serum to whole bacilli but, in contrast, had a very low protective titer for rats when compared with serum to whole bacilli. Serum to either of these fractions is incapable of neutralizing plague toxin. Both fractions will induce immunity in mice. Neither fraction is of value in producing immunity in guinea pigs.

Fraction II has never been obtained in a state approaching chemical or immunological purity. All preparations have been contaminated with Fraction I (IA and IB) in sufficient quantity to produce antibodies to Fraction I in rabbits. The best preparations had a toxicity (LD_{50}) for 20 g mice of 0.6 μg. However, it has been possible to prepare toxins free of Fraction I by serological technics. It was observed that plague bacilli grown at room temperature were fully toxic, but extracts of such bacilli contained very little Fraction I. It proved possible to remove the residual Fraction I by absorption with either Fraction IA or IB antisera. The resulting absorbed extract showed no decrease in toxicity, and produced antisera in rabbits capable of neutralizing plague toxin. However, this antiserum was devoid of protective value for mice, did not agglutinate antigenically complete plague bacilli to a significant titer, nor react with either Fraction IA or IB except to a very slight degree in sensitive ring tests. Its value in inducing immunity in mice and guinea pigs has not as yet been investigated.

From the *water-insoluble fraction* or "residue" only small amounts of protein can be extracted with mild alkalies. Anhydrous phenol liquefied with 10-15% acetone dissolves approximately 25% of the residue. Both fractions, the phenol-soluble as well as the insoluble one, contained the antigen which protects guinea pigs.

The 2 soluble atoxic antigens (Fractions IA and IB) undoubtedly represent the "en-

velope" antigen of Schütze[1] and others. They are probably present on the surface of the bacilli, because antisera prepared against these fractions will agglutinate the strains of plague bacilli thus far tested. Only one avirulent strain, TRU,[1] obtained from Dr. Harry Schütze, Lister Institute, London, England, is not agglutinated by Fraction I sera, although it is slightly agglutinated by sera prepared against whole plague bacilli. This strain appears to be devoid of these 2 fractions. The exact relationship between Fractions IA and IB is not clear. Serologically, they are almost identical. Thus far, the crystalline Fraction IB proved of slightly lower immunogenic value. It might be suggested that in the intact cell the normal antigen is the carbohydrate protein Fraction IA, and that the crystalline protein is an artifact formed by disaggregation of the molecule during the death of the cell and subsequent treatment. All attempts to alter Fraction IA short of denaturation have failed. It may be that plague bacilli contain an enzyme capable of converting Fraction IA to IB. Serologic studies have shown that Fraction I (IA and IB) is formed by the virulent strains tested, and the avirulent strain 1122 which forms a stable suspension in saline; avirulent strains showing salt instability produce only traces. These antigens are formed in quantity at 37°C; extracts of cells grown at room temperature contain relatively small amounts.

In view of the observed differences in response of the various laboratory animals to the different antigens of plague bacilli, it is considered advisable at the present time to use vaccines containing all of the antigens of *P. pestis* for prophylaxis of plague in man, and for the production of antiplague serum for therapeutic use.

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Liberation of Histamine and Heparin by Peptone from the Isolated Dog's Liver.*

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In previous reports[1-3] one of us has shown that several agents (antigen, *e.g.* horse serum in the sensitized animal, or peptone or Ascaris extracts) which *in vivo* produce a shock-like syndrome in the dog associated with a discharge of histamine and heparin from the liver, are unable to liberate significant amounts of these substances when perfused through the isolated liver, using Tyrode's solution as a vehicle. When citrated blood or heparinized blood is used as the perfusion fluid, detectable quantities of histamine and sometimes of heparin are discharged, but the total amounts that can be found in the perfusates are far smaller than those which are discharged in experiments *in vivo*. These

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