Serum Protein Changes Following Feeding of Parathion to Dogs. (23532)

MARTIN W. WILLIAMS, O. G. FITZHUGH, AND J. W. COOK (Introduced by E. P. Laug)
Divisions of Pharmacology and Food, Bureau of Biological and Physical Sciences, Food and Drug Admin., Washington, D.C.

The use of paper electrophoresis for fractionation of blood serum as well as other body fluids has given the pharmacologist a valuable tool for evaluation of some toxic manifestations of new pharmacological agents. Alterations in serum protein fractions, for example, whether relative or absolute, have been shown to accompany many pathological states. Marrack and Hock(5) in their review of serum proteins indicate that disease processes which involved liver parenchyma resulted in increase in gamma globulin and decreased albumin.

The purpose of this study was to determine whether a subacute non-lethal level of parathion, (0,0-diethyl 0-*p*-nitrophenyl thiophosphate), would produce any electrophoretically observable changes in blood serum proteins of the dog. Simultaneously blood cholinesterase levels were also studied.

Materials and methods. Five control cholinesterase determinations were made on plasma and red cells on 2 male and 2 female dogs over a 4-week period, by the electrometric method of Michel(6) as modified by Frawley(7). These animals were then placed for 9 weeks on a diet containing 100 ppm. of parathion in Purina Laboratory Chow. Results of plasma and red cell cholinesterase determinations are plotted in Fig. 1 at the end of 5th, 6th, 7th, and 13th weeks. During this 9-week parathion feeding, 9 blood samples were drawn from the 4 parathion treated dogs and an equal number from 5 control animals. Ten ml blood samples were drawn by external jugular vein puncture, placed in 12 ml centrifuge tubes containing 0.25 ml of 1% lithium oxalate and centrifuged at 2730 rpm for 15 minutes in standard Adams safety head fixed angle (52°) centrifuge (12.7 cm radius). The unclotted plasma was then separated from the cells and caused to clot by addition of 0.25 ml of 1% calcium chloride solution. The clotted plasma was then centrifuged again for 15 minutes and the supernatant serum separated from clot. Ten μl of this serum were placed on the "Spinco" sample striper and spotted alternately with control samples on 8 "Spinco" Whatman No. 3 mm filter paper strips. The procedure from this point on is adapted from that described by Williams *et al.*(8) using the hanging-strip type cell. The electrophoretic run was either for 16 hours at 3 milliamperes or for 6½ hours at 20-22 milliamperes constant current at 25-31°C. Under these conditions the average albumin migration from point of origin was 8.2 cm. The barbital buffer was pH 8.6 with an ionic strength of 0.05. Upon completion of experiment the strips were removed from the cell and placed horizontally in a flat glass dish which was then placed in a 100-110°C oven for 45 minutes. The strips were then placed in a 0.01% acidified bromphenol blue solution for 16 hours. Three rinsing baths in 2% acetic acid were followed by 3 to 5 minutes in 10% acetic acid 2% sodium acetate fixer solution. Drying was on filter paper at room temperature. The strips were scanned in a "Spinco" Analytrol with automatic integrator.

Results. The results of 18 samples of blood serum drawn over 9 weeks of the experiment, on 5 control and 4 parathion fed dogs (2 male and 2 females each) on parathion and 2 males and 3 females on control are compiled in Table I.

The results indicate a statistically significant relative increase in the β-globulin component and decrease in the albumin fraction as a result of feeding of parathion.

The results of cholinesterase determinations

TABLE I.

	Albumin	Globulins α_1	α_2	β	γ
Control	48.0	9.0	10.6	13.5	18.8
Parathion-fed (100 ppm.)	*34.15	7.9	11.6	*23.3	23.1

* Difference from control value is statistically significant.

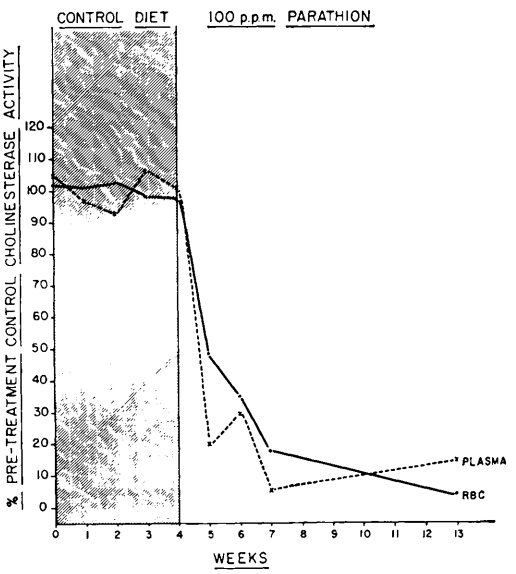

FIG. 1.

noted in Fig. 1 indicate the rapid decline in both plasma and red cell cholinesterase during the first 3 weeks of phosphate feeding. The terminal levels all below 20% of pre-treatment control indicate an injury which is also reflected in serum protein changes.

Fig. 2 shows typical serum sample patterns from both control and parathion fed dogs.

Discussion. As an index of toxicity, the study of serum protein changes offers promise. Marrack and Hock indicated(5) that the changes noted in serum proteins are not specific for any given pathological situation but yield an index to the overall constitutional state. The development of paper electrophoresis has provided the tool by which we may study the effects of toxic agents simply and rapidly without the necessity of elaborate and technically difficult older methods.

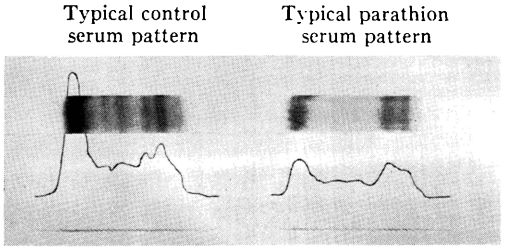

FIG. 2.

Much evidence to 1949(5) indicated that stressing agents, whether of functional, infectious or toxic nature, all produce rather typical changes in the blood serum picture. Whether we are dealing with syphilis, liver disease, malaria(1), liver ischemia(9), leg fractures(2), rickettsiosis, acute babesiosis (3) X-Ray(4) sulphur mustard, burns or turpentine(10), the resulting serum protein changes are essentially the same, namely, decreased albumin, both relatively and absolutely and a tendency for increased gamma and sometimes beta globulins. The present study further verifies that concept and points a new approach to the study of subacute functional damage from various toxic agents.

Summary. Serum electrophoretic patterns from dogs fed 100 ppm of organic phosphate parathion, indicate a relative decrease in level of albumin and increase in beta globulin. The significance of these findings is discussed.

1. Janssen, L. W., *Electrophoretic Studies on Serum Proteins,* North Holland Publishing Co., Amsterdam, 1951.

2. Cuthbertson, D. P., and Tompsett, S. L., *British J. Exp. Path.,* 1935, v16, 47.

3. Polson, A., and Malherbe, W. D., *Onderstepoort J. Vet. Res.,* 1952, v25, 13.

4. Muntz, J. A., Guzman-Barron, E. S., and Prosser, C. L., *Arch. Biochem.,* 1949, v23, 434.

5. Marrack, J. R., and Hock, H. J., *Clin. Path.,* 1949, v2, 161.

6. Michel, H. C., *J. Lab. and Clin. Med.,* 1949, v34, 1564.

7. Frawley, J. P., and Fuyat, H. N., *Agric. and Food Chem.,* 1957, v5, 346.

8. Williams, F. G., Pickles, E. G., and Durrum, E. L., *Science,* 1955, v121, 829.

9. Rappaport, A. M., Clarke, D. W., and Stewart, M., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 585.

10. Gjessing, E. C., Ludwig, S., and Chanutin, A., *J. Biol. Chem.,* 1947, v170, 551.

Received September 20, 1957. P.S.E.B.M., 1957, v96.