NOTES ON DECOMPOSITION OF PARATHION IN LIVING ORGANISMS*

II. SERUM PARATHION-SPLITTING ACTIVITY IN RELATION TO SERUM PROTEINS

IWAO IUCHI

Department of Clinical Pathology, Yamaguchi Medical School, Ube
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In the preceding paper[1] a detailed report was made on the presence in human and canine sera of a thermolabile substance capable of splitting parathion enzymatically. The changes of such activities were also studied in parathion poisoning produced in dogs.

Since the substance is thermolabile, contemplation will lead us to the following questions: (1) To what fraction of the serum protein does the parathion-splitting substance belong? And (2) Is there any similarity in the behavior in parathion poisoning between this substance and the serum proteins which are equally thermolabile?

In order to answer these questions two experiments were done, namely, (1) serum was subjected to paper electrophoresis and the paper strip was examined, and (2) the variation of the concentrations of serum total protein, albumin and globulin was compared with the alterations of the serum parathion-splitting activity in dogs which had received the injections of parathion, potassium diethyl thiophosphate (KDTP) or diethylchlorothiophosphate (DCTP: one of the precursor of parathion in its synthetic process). The experiment with DCTP was conducted for the reason that the commercial parathion may be contaminated with DCTP to the extent that its effect on the experimental animals can not be ignored.

MATERIALS AND METHODS

(1) Electrophoresis of the blood serum.[2,3,4] Fourteen to sixteen paper strips (3 cm in width; Toyo roshi No. 51) were moistened with a barbiturate buffer solution (pH = 8.9, $\mu = 0.1$) and installed in a paper electrophoresis apparatus. After sending a test current for 30 minutes 20λ aliquots of human blood serum were applied to each of the paper strips and subjected to electrophoresis (voltage gradient: 4 volt per 1 cm) for 15 hours. The paper strips were then removed from the apparatus and placed on a large glass plate separately. One of the strips was treated with a bromphenol blue solution in a conventional way to locate the protein fractions. Referring to this strip other papers were cut into five pieces according to the location of albumin and α_1, α_2, β and γ globulins. The pieces

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of strips of the same protein fractions were collected and cut into small fragments separately in five test tubes, to which were added 4 ml of bicarbonate-carbonate buffer solution (pH 9.37; the one used for the determination of parathion-splitting activity). The test tubes were allowed to stand at room temperature for about 2 hours to release the proteins from the paper, and then centrifuged to remove the paper fibers. Aliquots of 2 ml of each clear supernatant thus obtained were transferred into the other five test tubes separately, to which was added 0.5 ml of the emulsified parathion solution. The mixtures were incubated and the liberated p-nitrophenol were measured in exactly the same way as was specified in the determination of parathion-splitting activity. A portion of the same strip which was not in contact with protein was treated similarly to prepare the blank solution. The bromphenol blue-stained strip was applied to a densitometer to disclose the distribution of protein fractions.

(2) Variation of serum proteins in parathion-poisoned animals. Varying amounts of parathion (0.0 mg, 0,05 mg, 3.0 mg and 18.0 mg per Kg body weight) were injected sucutaneously to 12 dogs and blood samples were obtained from these animals by cardiac puncture. Amounts of KDTP and DCTP which were equivalent to these doses of parathion were given subcutaneously to two groups of dogs, each consisting of 12 animals. The same animals and the same blood specimens which were used in the previous paper also constituted the material in this study.

Serum protein concentration was read with a Hitachi's refractometric protein-meter.[5] Albumin and globulin were determined by Mizuta-Takahashi's salting-out (with 26 g per dl sodium sulfate) biuret method.[6]

RESULTS

The distribution of parathion-splitting activity in various protein fractions are presented in Table I.

TABLE I

Distribution of parathion-splitting substance (PSS) in fractionated blood serum protein (Human)

	Concentration of fractionated protein as expressed in percentage of total blood serum protein (%)			Area of paper strip sections (cm^2)			PSS activity as expressed in extinction at 397 mμ*		
	No.1	No.2	No.3	No.1	No.2	No.3	No.1	No.2	No.3
Albumin	53.5	52.2	48.2	78	66	86	116 (2.2)	163 (3.1)	109 (2.3)
α_1-globulin	5.1	6.8	5.1	31	40	39	94 (18.4)	128 (19.0)	115 (22.6)
α_2-globulin	7.5	5.2	10.1	31	46	39	65 (8.7)	115 (22.0)	67 (6.7)
β-globulin	17.7	11.0	11.2	31	53	51	33 (1.9)	60 (5.4)	89 (8.0)
γ-globulin	16.3	24.6	25.4	59	76	94	44 (2.7)	2 (0)	0 (0)
Blank	0	0	0	78	66	78	0 (0)	0 (0)	0 (0)
Conc. of serum protein (g/dl)	6.7	8.2	7.5						
A/G ratio	1.24	1.10	0.92						

*Figures in parenthesis refer to the parathion-splitting activity per one per cent of the individual fractionated proteins.

The averge values of the serum protein concentration, albumin and globulin shortly before the injections are listed in Table II for 3 groups, i.e. the parathion, DCTP and KDTP groups.

TABLE II

Average concentration of blood serum protein, albumin, and globulin, and albumin/globuiin ratio just before injection of drugs.

Drug	Blood serum protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
Parathion	6.86	3.22	3.64	0.89
Diethyl chlorothio-phosphate	6.80	3.23	3.57	0.91
Potasium diethyl thiophosphate	5.72	2.83	2.89	0.98

The fluctuation of serum protein concentration in parathion-poisoned dogs is shown in Figure 1. In the control animals the serum protein decreased slightly with the lapse of days. However, the dogs which received 3.0 mg and 18.0 mg per Kg of parathion exhibited a more pronounced reduction of serum protein,

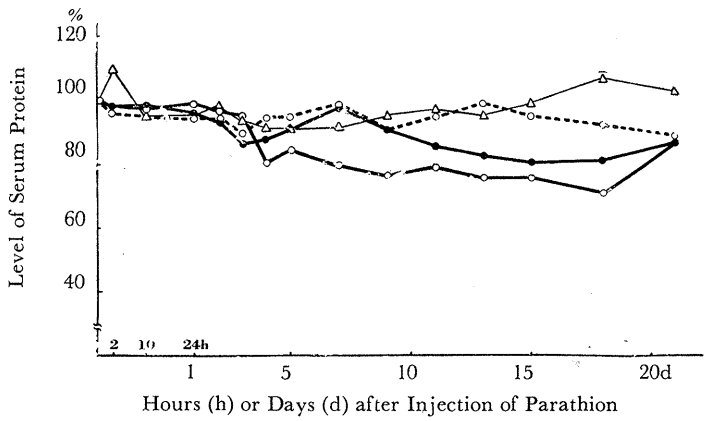

Fig. 1. Vicissitude of serum protein level in parathion-poisoned dogs.
○·····○ : Control, △——△ : 0.5 mg, ●——● : 3.0 mg,
○——○ : 18 mg per kg parathion.

particulary after one week from the commencement of the experiment. The reduction was as much as 20 to 30 per cent of the original serum protein concentration after the second week. Apart from the occasional transitory decreases found in the animals receiving 0.5 mg and 18.0 mg parathion-equivalent of the drug no remarkable change in serum protein concentration was observed in the DCTP group (Figure 2). The KDTP group showed almost constant levels of serum protein throughout the experiment with the exception of control animals

which peculiarly presented a gradual reduction (Figuer 3). In the parathion group the serum albumin concentration of the poisoned animals rose slightly over that of the control animals for three days after injection and then fell slowly until 70 to 80 per cent of the original level was reached (Figure 4). The DCTP group seemd to show no appreciable change in serum albumin concentration as compared with the control animals, but definitive conclusion was to be withheld because of considerable individual variations (Figure 5). In the KDTP group the drug appeared to

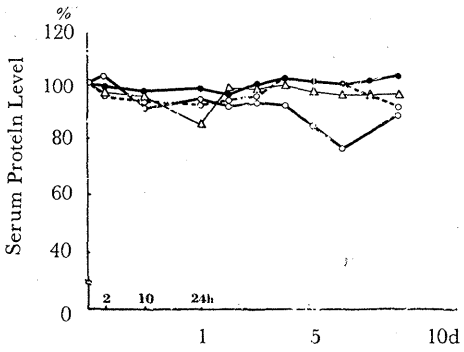

Fig. 2. Vicissitude of serum protein level in dogs injected with diethyl chlorothiophosphate.
○·····○ : Control, △——△ : 0.352 mg, ●——● : 2.14 mg, ○——○ : 12.66 mg per Kg DCTP.

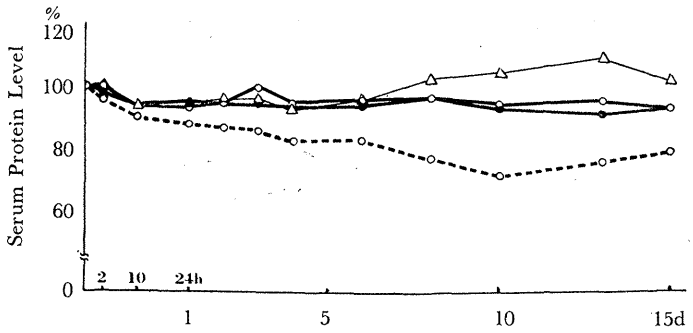

Fig.3. Vicissitude of serum protein level in dogs injected with potassium diethylthiophosphate.
○·····○ : Control, △——△ : 0.357 mg, ●——◐ : 2.1445 mg, ○——○ : 12.870 mg per Kg KDTP.

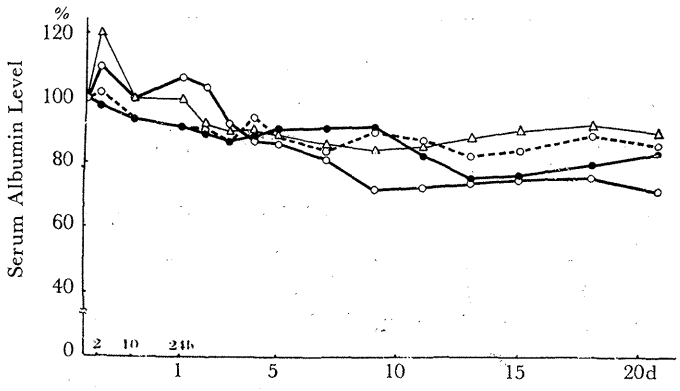

Fig.4. Vicissitude of serum albumin level in parathion poisoned dogs.
○·····○ : Control, △——△ : 0.5 mg, ●——◐ : 3.0 mg, ○——○ : 18 mg per Kg parathion

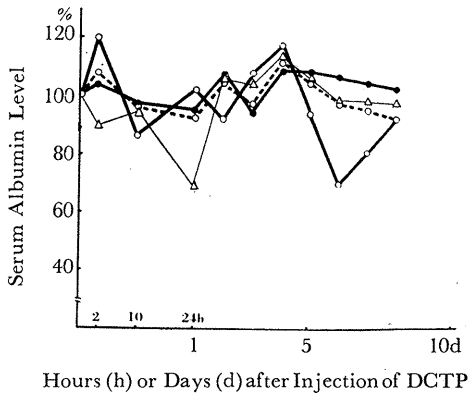

Fig. 5. Vicissitude of serum albumin level in dogs injected with diethyl chlorothiophosphate.
○·····○ : Control, △——△ : 0.35 mg, ●——● : 2.14 mg, ○——○ : 12.66 mg per Kg DCTP.

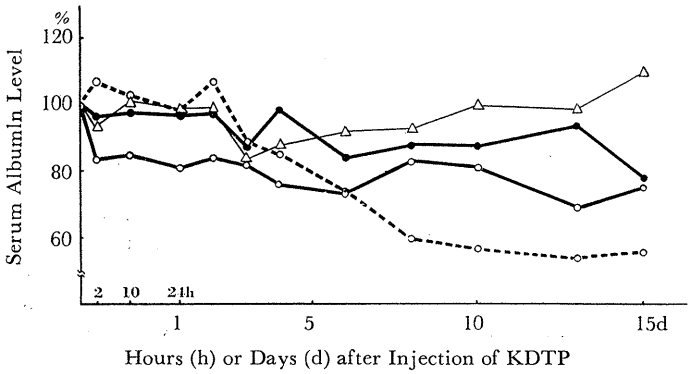

Fig. 6. Vicissitude of serum albumin level in dogs injected with potassium diethyl thiophosphate.
○·····○ : Control,
△——△ : 0.357 mg,
●——● : 2.144 mg,
○——○ : 12.870 mg per Kg KDTP.

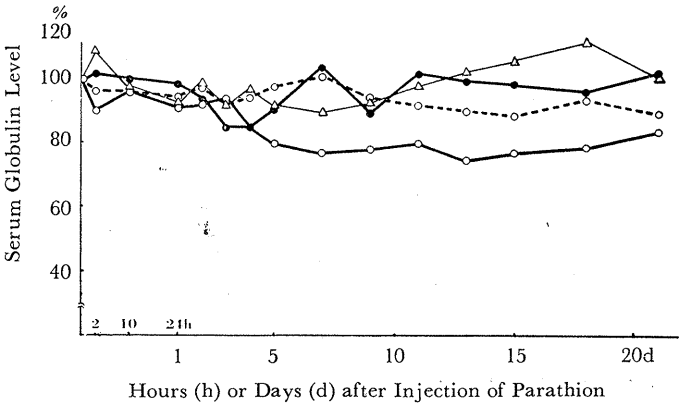

Fig. 7. Vicissitude of serum globulin level in dogs injected with parathion.
○·····○ : Control,
△——△ : 0.5 mg,
●——● : 3.0 mg,
○——○ : 18.0 mg per Kg parathion.

exert no signicant influence on the serum albumin level. The drop of albumin concentration was more marked in the control animals than in those receiving KDTP (Figure 6).

Serum globulin exhibited a gradual decrease in the parathion group (Figure 7)

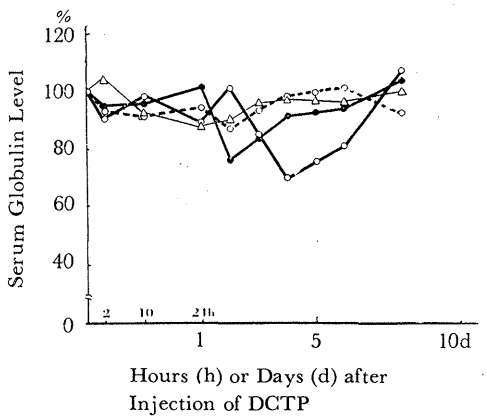

Fig. 8. Vicissitude of serum globulin level in dogs injected diethyl chlorothiophosphate.
○·····○ : Control, △——△ : 0.352 mg, ●——● : 2.14 mg, ○——○ : 12.66 mg per Kg DCTP.

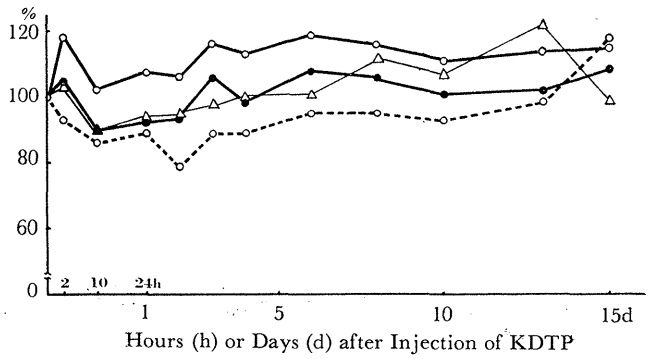

Fig. 9. Vicissitude of serum globulin level in dogs injected with potassium diethylthiophosphate.
○·····○ : Control,
△——△ : 0.357 mg,
●——◑ : 2.144 mg,
○——○ : 12.870 mg
per Kg KDTP.

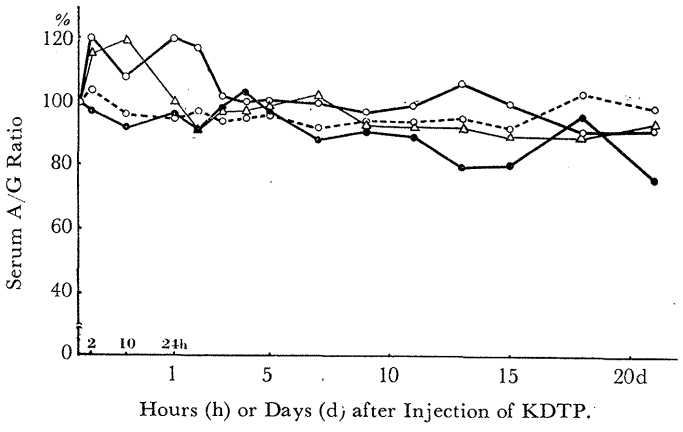

Fig. 10. Vicissitude of serum A/G ratio in dogs injected with potassium diethylthiophosphate.
○·····○ : Control,
△——△ : 0.357 mg,
●——◑ : 2.144 mg,
○——○ : 12.870 mg
per Kg KDTP.

and the DCTP group (Figure 8), while a slight tendency toward increase in globulin was noted in the KDTP group (Figure 9).

In the parathion group there was a transitory slight rise in albumin-globulin ratio for the first few days but in the later period of observation the ratio remained

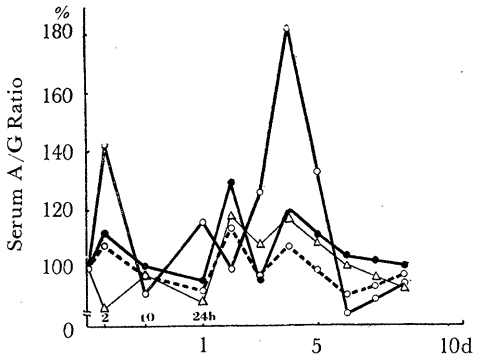

Fig. 11. Vicssitude of serum A/G ratio in dogs injected with diethyl chlorothiophosphate.
○·····○: Cotrol, △——△: 0.352 mg, ●——●: 2.14 mg, ○——○: 12.66 mg per Kg DCTP.

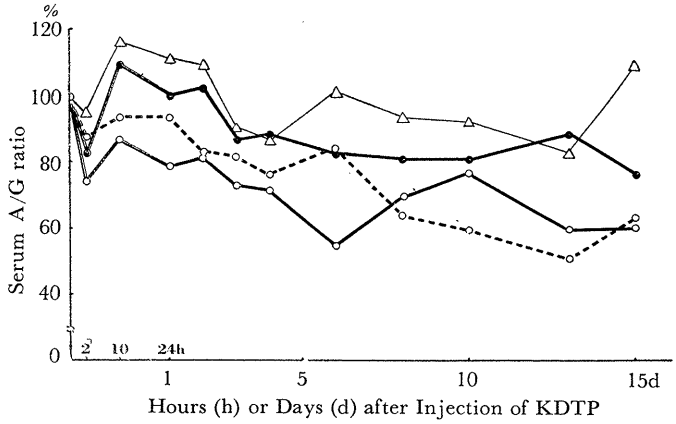

Fig. 12. Vicissitude of serum A/G ratio in dogs injected with potassium diethy!thiophosphate.
○·····○: Control,
△——△: 0.357 mg,
●——●: 2.144 mg,
○——○: 12.870 mg per Kg KDTP.

at almost constant levels (Figure 10 and 11). On the other hand, in the KDTP group the albumin globulin ratio showed a continuous decrease after an early transitory rise (Figure 12).

DISCUSSION

It is apparent from Table I that the parathion-splitting activity is distributed in albumin and α_1, α_2 and β globulin with its peak in α globulin. Alpha-globulin is accordingly thought to play a major rôle in the decompostion of parathion.

According to Aldridge[7,8] E 600 esterase which decomposes E 600 (paraoxon, an analogue of parathion) was demonstrated in the globulin fraction by Cohn's ethanol fractionation, whereas it was found in the albumin fraction when the serum was salted out with ammonium sulfate. Such discrepancy will be readily explained, if E 600 esterase is distributed in α gloulin.

Hiraki[9,10] stated in his extensive clinical study on human parathion poisoning that, in the severely poisoned patient, the serum protein increased on the first day of poisoning, decreased during the 2nd to 5th day, rose again the 8th to 13th

day and remained constant after the third week. In the moderately poisoned cases serum protein presented no remarkable change on the first day, decreased during the second to fifth day, and then gradually the original level of serum protein was restored. In mild poisoning, no appreciable changes in serum proteins were seen. As regards the albumin-globulin ratio, he observed a transitory rise as a result of increased albumin associated with decreased globulin on the first day of poisoning.

The early and temporary rise in the albumin-globulin ratio in the parathion-poisoned dogs may be similar in nature to the transitory increase in albumin globulin ratio found in human material. However, the late pattern of total protein, albumin, globulin and albumin-globulin ratio would be altered by impaired nutrition by cage feeding to such an extent that any definitive conclusion could hardly be warranted. The results with DCTP and KDTP are to be interpreted similarly. It seems reasonable to conclude, therefore, that parathion, DCTP and KDTP will not directly affect the pattern of serum proteins.

It is very interesting that like serum parathion-splitting activity the serum protein did not exhibit remarkable change throughout the course of canine parathion-poisoning.

Conclusion

1. Serum parathion-splitting substance is distributed in albumin, and α_1, α_2 and β globulins with its major portion in α_1 and α_2 globulin fractions.
2. Injection of parathion, DCTP to dogs induced no appreciable change in the concentration of serum protein, albumin, globulin or in the albumin-globulin ratio. The behavior of these serum constituents in parathion poisoning appears to be similar to that of the serum parathion splitting substance.

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