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NOTES ON THE DECOMPOSITION OF PARATHION IN LIVING ORGANISMS

III. SERUM PARATHION SPLITTING ACTIVITY AND SERUM ALKALINE PHOSPHATASE

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In the last five years several enzymes which decompose organophosphorus compounds have been discovered. They are *Mazur*'s esterase[1] for diisopropyl fluorophosphate (DFP), A esterase[2,3] for paraoxon (diethyl p-nitrophenyl phosphate, E 600), *Kubistova*'s enzyme[4], and alkaline phosphatase[5] for tetraethyl pyrophosphate (TEPP). It has been postulated that organophosporus compounds are also subject to contact decomposition with various metal ions, such as Ca^{++}, Pd^{++}, Au^{+++}, Ag^{+}, Ni^{++}, Co^{++} and Zn^{++}[6].

As for parathion, it has hitherto been thought that cholinesterase is responsible for its decomposition[7,8,9]. According to this theory, while cholinesterase is inactivated by parathion (as would be expected), it also adversely effects parathion by chemical combination and decomposes it in a certain length of time.

Our experiment detailed in the preceding papers[10,11] revealed the presence of an enzyme-like parathion-splitting substance in human and canine blood serum. It was also found in this experiment that there was no correlation between the parathion-splitting activity and the cholinesterase. Interestingly enough, the decomposition of parathion by blood serum was greatest at the optimum pH of serum alkaline phosphatase. The identity of serum parathion-splitting substance with serum alkaline phosphatase has, consequently, come up as a possibility.

In order to resolve this problem an animal experiment was designed as follows: (1) Serum alkaline phosphatase was determined concurrently with the estimation of the serum parathion-splitting activity in dogs which had received the injection of parathion or potassium diethyl thiophosphate (an analoguous compound of parathion) so that the correlation between the two enzymes might be assessed. (2) The activators and inhibitors of the serum parathion-splitting substance were examined to compare them with those of serum alkaline phosphatase. Several additional experiments were also performed to differentiate the serum parathion-splitting substance from the enzymes related to the other organophosphorus compounds.

METHODS

The dogs used in this experiment were the same animals which were injected

with parthion or potassium diethylthiophpsphate (KDTP) and used in the previous papers.[10,11] Serum parathion-splitting activity was determined by the author's procedure,[10] and serum alkaline phosphatase (AP) activity by a modified *Shinowara-Jones-Reinhart*'s procedure[12,13] employing glycerophosphate as substrate in bicarbonate-carbonate buffer solution (pH 10.0).

RESULTS

The average levels of serum alkaline phosphatase and serum inorganic phosphorus previous to the injection of drugs were 4.5 *Bodansky* units per 100 ml and 5.3 mg per 100 ml, respectively. In this paper the variation of alkaline phospha-

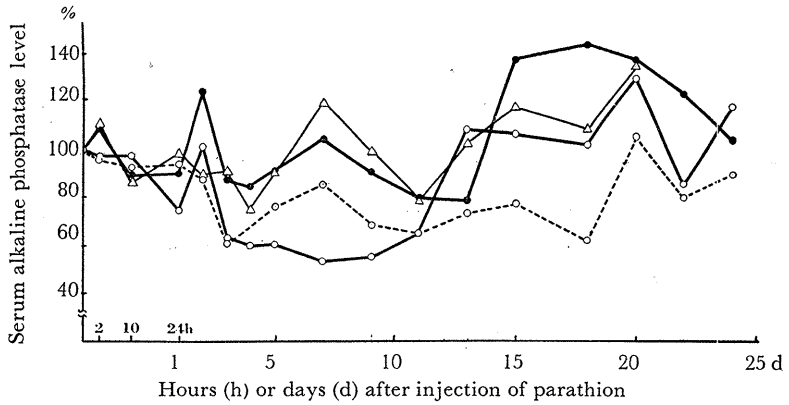

Fig. 1. Vicissitude of serum alkaline phosphatase level in dogs injected with parathion. ○·····○: Control, △——△: 0.5 mg, ●——●: 3.0 mg, ○——○: 18.0 mg per kg parathion.

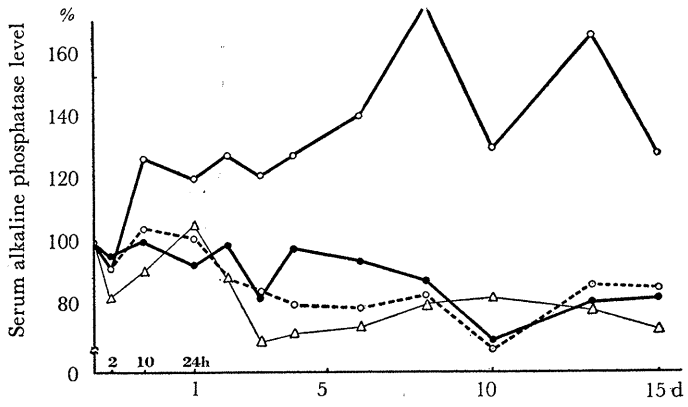

Fig. 2. Vicissitude of serum alkaline phosphatase level in dogs injected with potassium diethylthiophosphate. ○·····○: Control, △——△: 0.357 mg, ●——●: 2.144 mg, ○——○: 12.870 mg per kg KDTP.

tase and inorganic phosphorus will be expressed in percentage of these averages which are taken as 100 per cent.

Figure 1 represents the variation of serum alkaline phosphatase in the dogs received parathion. The group of dogs injected with 0.5 and 3.0 mg per kilogram of parathion showed increased alkaline phosphatase activities, as much as 20 per cent higher than those of the control animals, for the period of from the first to the tenth day. The rise in serum alkaline phosphatase became more marked after the eleventh day until it reached 150 to 200 per cent level on the seventeenth or eighteenth day. In the group injected with 18 mg per kilogram of parathion there was no sign of the increase of serum alkaline phosphatase before the eleventh day, when the enzyme began to rise over the control level.

The variation of serum alkaline phosphatase was not very remarkable in the

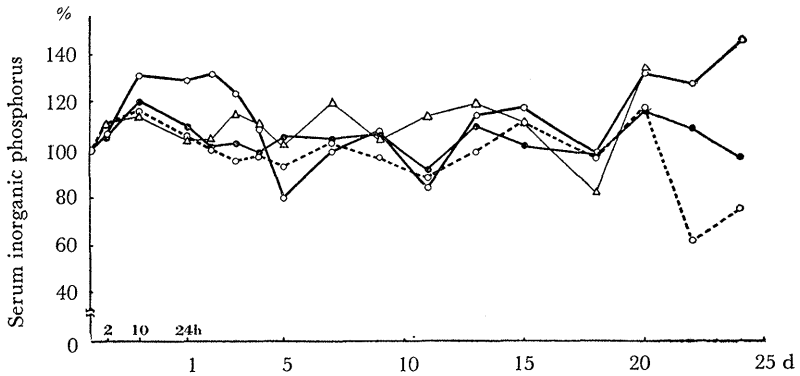

Fig. 3. Vicissitude of serum inorganic phosphorus in dogs injected with parathion. ○·····○: Control, △——△: 0.5 mg, ●——●: 3.0 mg, ○——○: 18.0 mg, per kg parathion.

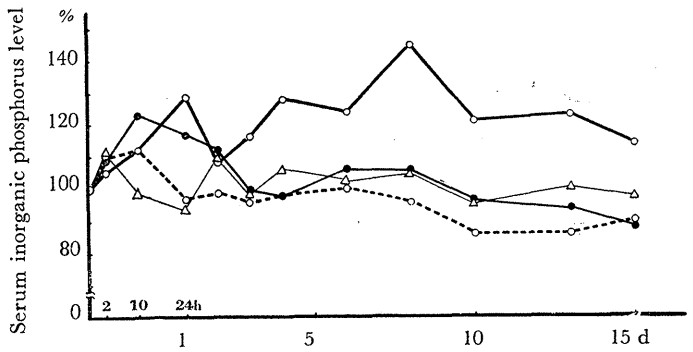

Fig. 4. Vicissitude of serum inorganic phosphorus in dogs injected with potassium diethylthiophosphate. ○·····○: Control, △——△: 0.357 mg, ●——●: 2.124 mg, ○——○: 12.870 mg

dogs receiving relatively small doses (0.5 and 3.0 mg per kg) of KDTP. In constrast, the animals injected with a large dose (18mg per kg) exhibited distinctly higher levels throughout the period of observation (Figure 2).

Serum inorganic phosphorus increased slightly in the animals poisoned with parathion (Figure 3), and a similar or greater increase was also observed in the KDTP group (Figure 4).

In sharp contrast to alkaline phosphatase and inorganic phosphorus, the serum parathion-splitting activity was not affected to any appreciable extent by the injection of parathion or KDTP (Figure 5).

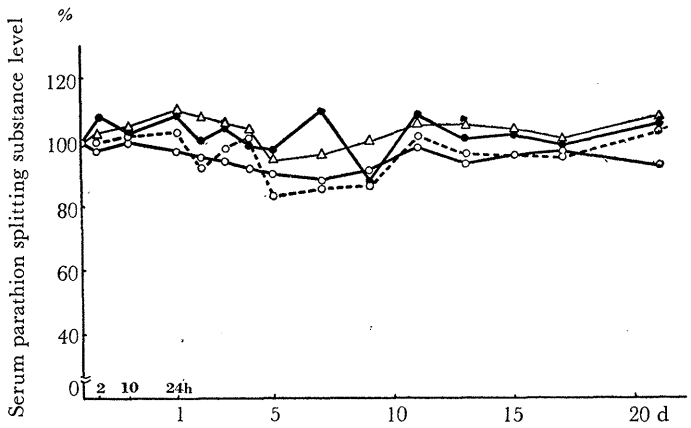

Fig. 5. Vicissitude of serum parathion splitting substance level in dogs injected with parathion ○·····○: control, △——△: 0.5 mg ●——●: 3.0 mg, ○——○: 18.0 mg per kg parathion

TABLE I

Inhibitors of cholinesterase (ChE), alkaline phosphatase (AP) and serum parathion splitting substance (PSS)

	ChE	AP	PSS
Parathion	+	−	−
Serum activity pretreated with parathion	+	−	−
Eserine	+	−	−
Sodium fluoride	+	+	−
Nickel sulfate	(−)	(−)	+

+ : Inhibition, − : No inhibition

Table I shows the result of the study on inhibition of serum parathion-splitting substance, cholinesterase and alkaline phosphatase. It was noticed that serum parathion-splitting activity was inhibited neither by eserine, an inhibitor of cho-

linesterase, nor by fluoride, an inhibitor of alkaline phosphatase, but was inactivated by nickel sulfate.

The activators of serum parathion-splitting substance were listed in Table II. Thiosulfate, azan and fructose were the activators worthy of mention.

TABLE II

Activators of serum parathion splitting substance and its activation rate (%)

Dilution of activators	1×	5×	25×	125×
50% Glucose	7.5	5.0	0.9	0.1
20% Fructose	9.7	8.0	7.0	6.2
Azan (Sodium 8-Azaguanine)	4.6	3.7	3.6	—
10% Sodium thiosulfate	15.8	11.1	8.8	8.5
Banthionin	5.8	6.7	3.6	—

DISCUSSION

It is obvious from the difference between serum parathion-splitting substance and serum alkaline phosphatase in the behavior in posioning and in the conditions and activation of activities that they are not the same components. The range of optimum pH is not specific enough to identify both substances because a same optimum pH could be a sheer coincidence.

It was also pointed out in the previous papers that there was a remarkable difference between the serum parathion-splitting substance and serum cholinesterase, particularly in the pattern of activity alterations in parathion poisoning. The observation that eserine, a strong inhibitor to cholinesterase, failed to exert any influence upon serum parathion-splitting activity provides additional evidence to disprove identity of these enzymes.

It is of interest that sodium thiosulfate which proved in our experiment to be the most potent activator of serum parathion-splitting substance has been ranked by some authors among the therapeutic agents for human parathion poisoning.[9] Presumably sodium thiosulfate benefits the detoxication of parathion by enhancing the parathion-splitting activity.

Aldridge's A esterase may be the best-studied of the enzymes related to organophosphorus compounds.[1,2] It splits paraoxon, an analogue of parathion, and phenyl acetate. Its optimum pH is from 7.4 to 7.6. It is inhibited by copper sulfate, p-chloromercuribenzoic acid and sodium iodoacetate. Serum parathion-splitting substance exhibits its maximum activity at a higher pH range (pH 8.6–8.8) without inactivation by copper ion. It is therefore different from A esterase.

Unfortunately, the serum parathion-splitting substance could not be compared

with *Mazur*'s esterase[1] or *Kubistova*'s enzyme,[4] since the literature concerning these enzymes was not avilable in our library.

It will be worthy of notice that there was a tendency of increase in serum alkaline phosphatase in the dogs which received the injection of KDTP. Since KDTP, being a phosphodiester, will be decomposed by a phosphoesterase, its presence in the blood will presumably stimulate tissues to produce increased amounts of phosphoesterases, to which belongs alkaline phosphatase. Parathion is decomposed by serum parathion-splitting sunstance into p-nitrophenol and diethylthiophosphate. The diethylthiophosphate thus liberated will similarly promote increased production of phosphoesterases including alkaline phosphatase because it is a phosphoester. Failure of increase in serum alkaline phosphatase in the dogs injected with a large amount of parathion will probably be accounted for by deterioration of tissues due to excessive parathion and hence lack of tissue phosphoesterase production.

SUMMARY AND CONCLUSION

The variation of serum parathion-splitting activity and serum alkaline phosphatase in the dogs poisoned with varied amounts of parathion and KDTP was studied to examine the similarity of these enzymes. Their inhibitors and activators were compared for the same purpose. The following are the conclusions:

1. Serum parathion-splitting substance is not identical with serum alkaline phosphatase. Both have the the same optimum range of pH, but in the former is not inactivated by fluoride which is a potent inhibitor to the latter. The parathion-splitting activity is not affected and remains rather constant in parathion-poisoned dogs presenting a distinct contrast to serum alkaline phosphatase which shows a considerable increase under the same conditions.

2. Serum parathion-splitting substance is neither identical with cholinesterase nor A esterase. Their behavior in parathion or KDTP poisoning, optimum pH, inhibitors and activators are different.

3. There is a rise in serum alkaline phosphatase activity in the dogs injected with parathion or KDTP. Deduction is made that diethylthiophosphate which is a partial degradation product of these drugs stimulates the tissues to produce increased amounts of alkaline phosphatase.

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