NOTES ON THE DECOMPOTITION OF PARATHION IN LIVING ORGANISMS

IV. DISTRIBUTION OF PARATHION-SPLITTING SUBSTANCE IN VARIOUS ORGANS: COMPARISON WITH CHOLINESTERASE AND ALKALINE PHOSPHATASE

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(Received May 1, 1957)

Human and canine blood sera contain an enzyme-like substance which liberates p-nitrophenol from parathion at an alkaline pH. Its chemical properties and behavior in canine parathion poisoning have been reported in the previous paper.[1] Inasmuch as the blood functions as a vehicle for the substances metabolized in the organs, the presence of parathion-splitting substance the blood serum will be interpreted as an indication that organs are producing and releaseing this substance into the blood stream. Accordingly, the distribution of parathion-splitting substance in various organs absorbed our interest. Such a study would eventually throw some light on the nature of this substance.

In this paper, the tissue (or organ) distribution of parathion-splitting substance was studied with dogs. Alkaline phosphatase and cholinesterase were measured concurrently in comparison with the parathion-splitting substance since these are the enzymes which some authors[2,3] claim to have the potential to decompose parathion.

METHODS

Four male and six female dogs (weighing 7 kg on an average) were sacrificed and their organs (the brain, lungs, heart, liver, spleen, pancreas, stomach, small intestine, kidneys, bone marrow, lymphnodes and bile in the gall bladder) and blood were taken for the measurement of parathion-splitting activity.

Pieces of tissues weighing about 5g (the stomach and small intestine were scraped with a glass edge from the muscle and serous layers) were excised from the organs and cut into 40μ frozen sections by a freezing microtome. The sections (approximately 4 g together) were weighed accurately in a chemical balance, transferred into a test-tube homogenizer with an equal amount of physiological saline and ground. Two-tenths g of 50 per cent tissue homogenates thus prepared was measured for parathion-splitting activity by the procedure[1] for the estimation of serum parathion-splitting substance.

For the measurement of alkaline phosphatase activity a modified *Shinowara-Jones-*

Reinhart's procedure[4,5] with glyceropohsphate as substrate in bicarbonate-carbonate buffer solution (pH 10.0) was employed after adequately diluting the 50 per cent homogenates with physiological saline. Marked variations of activities in the organs made it necessary to prepare varying dilutions from 0.5 to 10.0 per cent of the homogenate. Two male and six female dogs were used for the study of alkaline phosphatase.

Cholinesterase activity of the organs were determined by *Hestrin*'s colorimetric method.[6] Two female dogs were sacrificed and 10 per cent homogenates of the tissues were prepared for the determination of cholinesterase.

RESULTS

As clearly seen in Table I which summarizes the study on tissue distribution of parathion-splitting activity, the blood serum had the highest activity of parathion decomposition. The liver and the lungs were also rich in parathion-splitting substance, occupying the second and the third place, respectively. They were followed by the bone marrow, kidneys, intestine, spleen, lymphnodes, stomach, pancreas, heart, brain, and bile, in the order listed.

Table I. Parathion-splitting activity of various tissues (dog; γPNP/g•hr)

No. of dogs	1	2	3	4	5	6	7	8	9	10	Average
Sex (Male, Female)	F	F	F	F	M	M	M	F	M	M	
1 Blood serum	260	282	296	184	349	289	292	258	149	215	257.4
2 Liver	280	194	348	88	318	152	miss	224	198	206	200.6
3 Lung	190	220	448	100	116	88	56	60	64	22	136.4
4 Bone marrow	182	miss	216	80	122	18	196	50	132	168	130.0
5 Kidney	112	74	234	76	168	70	90	152	92	80	103.6
6 Small intestine	70	74	74	110	132	80	20	36	70	92	75.8
7 Spleen	58	34	88	48	114	54	54	8	118	44	62.0
8 Lymph node	15	15	88	30	212	50	12	32	10	76	53.8
9 Gastric mucosa	50	80	66	62	36	24	74	28	20	60	50.0
10 Pancreas	—	50	154	44	0	8	21	48	8	50	38.4
11 Bile	158	66	34	38	30	18	8	0	10	10	37.4
12 Brain	0	14	34	0	0	0	12	32	8	4	10.4
13 Heart	6	0	0	0	15	0	32	0	0	0	6.8

Tabel II and Figure 1 show the comparison of parathion-splitting activities in various tissues as expressed in percentage of the serum activitity. The liver, lungs bone marrow and the kidneys, exhibited 80, 50, 50 and 40 per cent of the serum

parathion-splitting activity, respectively. The brains and the heart had so little activities that they were not quite 5 per cent of the liver.

Table II Parathion-splitting activity of various tissues as expressed in the percentage of the activity of blood serum (dog)

No. of dogs		1	2	3	4	5	6	7	8	9	10	Average
Sex (Male, Female)		F	F	F	F	M	M	M	F	M	M	
1	Blood seum	100	100	100	100	100	100	100	100	100	100	100
2	Liver	107	69	122	44	87	52	miss	86	133	88	79.0
3	Lung	74	78	158	54	32	28	19	25	44	9	52.0
4	Bone marrow	70	miss	75	43	35	6	67	20	90	73	48.0
5	Kidney	1	27	83	41	46	24	31	59	60	34	40.6
6	Samll intestine	27	27	26	64	36	28	7	14	48	40	32.0
7	Spleen	22	13	31	26	32	19	19	3	53	19	23.8
8	Lymph node	6	6	30	16	58	19	4	8	7	32	17.6
9	Gastric mucosa	19	28	24	33	10	9	25	11	14	26	19.8
10	Pancreas	1	18	54	24	0	3	8	19	6	21	15.4
11	Bile	61	24	14	20	8	6	3	0	7	4	14.6
12	Brain	0	5	12	0	0	0	4	13	5	2	4.1
13	Heart	3	0	0	0	9	0	12	0	0	0	2.3

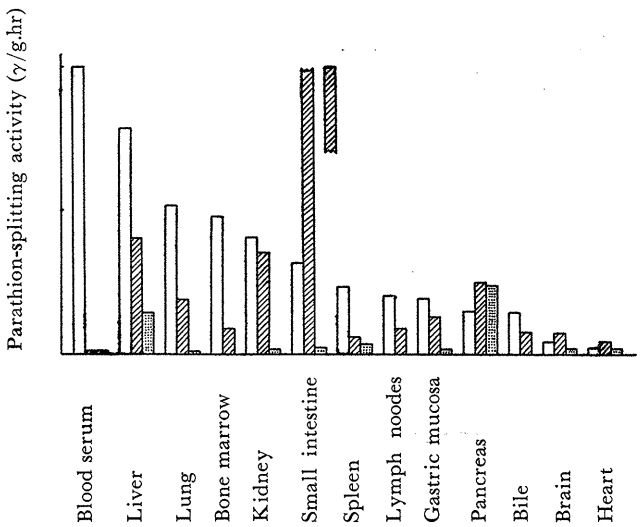

Fig. 1. Parathion-splitting activity of various tissues (p-nitrophenol γ per g. hour). White column PSS activity. Shaded column: Alkaline phosphatase activity. Dotted column·· Cholinesterase activity.

Table III presents the distribution of the parathion-splitting activity in various organs as calculated from the average activities on weight basis. In this table it is again confirmed that the serum, liver and lungs are the major organs of parathion decomposition. The brains and heart, the vitally important organs, contain the least amounts of parathion-splitting substance.

Table III. Parathion-splitt ing activity of various organs of a dog weighing 7.0kg.

		av. weight of organs (g)	av. PSS activity of tissues (γ/ml.)	av. PSS activity of total organs (γ/hr)
1	Blood serum	360	254.7	92,500
2	Liver	244	200.6	48,800
3	Lung	102	136.6	13,900
4	Bone marrow	—	130.0	—
5	Kidney	64	103.6	6,640
6	Small intestine	—	75.8	—
7	Spleen	23	62.2	1,430
8	Lymph node	—	53.8	—
9	Gastric mucosa	80	50.0	4,000
10	Pancreas	21	38.4	805
11	Bile	—	37.4	—
12	Brain	53	10.4	550
13	Beart	79	16.8	534
	Total			169, 162 γ, being equivalent to 354 mg of parathion.

The tissue distribution of alkaline phoaphatase and cholinesterase is summarized in Table IV. Figure 1 which compares the activities of parathion-splitting substance and these enzymes in various tissues clearly indicates the difference of tissue distribution.

Table IV. Distribution of alkaline phosphatase (AP) and cholinesterase (ChE) in various tissues (dog)

	Tissues	AP (mgP/g)	ChE (μMol/g)
1	Blood serum	8.1	87.0
2	Liver	325.0	1272.0
3	Lung	154.0	88.0
4	Bone marrow	67.2	—
5	Kidney	287.4	96.0
6	Small intestine	1629.4	192.0
7	Spleen	45.8	275.0
8	Lymph node	70.4	—
9	Gastric mucosa	103.2	151.0
10	Pancreas	203.2	2117.0
11	Bile	59.0	—
12	Brain	57.4	165.0
13	Heart	33.0	165.0

Discussion

The absence of similarity in the tissue distribution between parathion-splitting substance and alkaline phosphatase or cholinesterase provides further evidence for the view that parathion-splitting substance is entirely different from these enzymes. It is apparent from the data presented in the last part of this paper that the blood (serum), liver and lungs play the most important role in the detoxication. Likewise, the kidneys and perhaps bone marrow may be significant organs in this respect.

The fact that the parathion-splitting substance is contained most abundantly in blood serum may deserve special consideration, because it makes a sharp contrast to alkaline phosphatase and cholinesterase. These enzymes are present in the blood serum in far less amounts than in other organs. It will therefore be expected that the blood gets the supply of these enzymes from solid organs such as the liver, pancreas, small intestine, brain and heart. However, similar assumption may not be feasible in the case of parathion-splitting substance, because this substance is less abundant in the liver, lungs and other organs than in the blood. Such a gradient of distribution does not justify solid organs as the supplier of the enzyme to the blood.

The parathion decomposing capacity of dogs seems to be tremendous, because the theoretical figures of such capacity of the tested organs in the form of homogenate total approximately 354mg equivalent (with 7 Kg animal) at pH 8.8 in an hour period (Table III). As stated in the previous papers the activity of parathion-splitting substance at the blood pH level (7.4) is about one-third of that at pH 8.8. Accordingly, the capacity of parathion detoxication for a living dog of 7 Kg will be estimated at $1/3 \times 354$mg or 118mg of parathion for an hour. It is therefore expected that when a total of 126mg or 18mg per Kg of parathion was injected to a dog weighing 7 Kg, the drug would be decomposed completely within 65 minutes.

This conjecture may not be acceptable, because the fall of serum cholinesterase, an indicator of intoxication, lasts a considerably long time in parathion poisoning. However, evidence for the rapid disposal of parathion by living organisms is accumulating in the literature. From the result of his animal experiment with S^{35} -labeled parathion, *Jensen*[7] came to a conclusion that the parathion administered cutaneously or intravenously is not stored up in blood or other organs, since it is rapidly excreted in the urine in decomposed forms or unchanged. *Gar*[8] injected 6 to 10 mg of P^{32} -labeled parathion per Kg (almost fatal dose) to rabbits through the ear vein to study its decomposition and remarked that it was completely decomposed within thirty to sixty minutes. Our observation that the major part of p-nitrophenol was excreted in the urine before the third day in canine parathion poisoning will also connote its rapid decomposition in the body. It is therefore

probable that parathion is decomposed rapidly in the living organism by parathion-splitting substance, even if the rate of break down may not be so fast as estimated above.

Rapid decomposition of parathion in the body deserves special attention in the treatment of parathion poisoning, and delayed administration of any parathion decomposing agent may prove ineffective. Unless immediate detoxication was effected, the correction of disturbances of the autonomic nervous system and other organs as a result of cholinesterase inhibition would be more fruitful than the attempt to decompose the toxin still remaining in the body.

SUMMARY AND CONCLUSION

The tissue (or organ) distribution of parathion-splitting substance was studied in dogs and was compared with that of alkaline phosphatase and cholinesterase. The conclusions derived from this experiment are:

1. The blood serum showed the greatest parathion-splitting activity (257.4γ PNP/g. hour). The liver, the lungs and the bone marrow were also the important tissues which decomposed parathion. There was no significant parathion-splitting activity in the brain and the heart.

2. Blood (plasma) is an organ which plays the greatest role in the detoxication (decomposition) of parathion, and the liver is the second important.

3. There was no similarity in tissue distribution between the parathion-splitting substance and cholinesterase or alkaline phosphatese. The parathion-splitting substance is not identical either with alkaline phosphatase or cholinesterase.

Grateful acknowledgements are due to Dr. Takaoki Miyaji for his valuable help in the performanece of this experiment as well as to Prof. Susumu Shibata for his kind advice in the preparation of manuscript.

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