PECULIARITIES OF THE DISTRIBUTION OF THE HYDROCHLORIDE OF THE DIETHYLAMINOETHYL THIOESTER OF DIPHENYLHYDROXYTHIOACETIC ACID IN THE BODY

E. K. Balashova

From the Biochemical Laboratory (Head – Prof. V. I. Rozengart) of the All-Union Research Sanitary Chemical Institute (Director – S. N. Golikov), Leningrad

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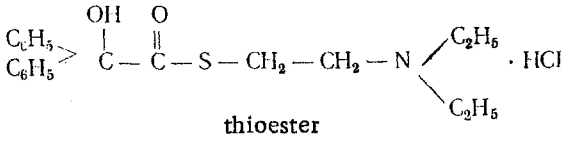
A. M. Kats [1] investigated the character of the distribution of the hydrochloride of the diethylaminoethyl thioester of diphenylhydroxythioacetic acid (thioester) in the body in various animals and studied the routes and the rate of its excretion from the body after intramuscular injection:



thioester

The aim of the present work was to study the peculiarities of the distribution of thioester in the body when administered in different ways, and to investigate the stability of its interaction with individual biochemical structures in the tissues.

EXPERIMENTAL METHOD

For this investigation, thioester labeled with radioactive sulfur (S^{35}) was synthesized.*

As experimental animals cats weighing 2-3 kg were used. In the work, the preparation was injected in various ways: intravenously (into the femoral vein) and intra-arterially (into the carotid and vertebral arteries). The preparation was injected as a 1% aqueous solution in a dose of 1-5 mg/kg. After predetermined intervals of time the animals were decapitated, the organs extracted, cleaned of blood and fat and homogenized in alkali by heating.

In fractionation experiments after the intravenous injection of thioester or the direct addition of the preparation to the finely chopped tissue, the organs were frozen in liquid oxygen and ground to the consistency of a fine powder, after which weighed samples were treated with 5% trichloroacetic acid (TCA). These were centrifuged and the centrifuge washed 10 times with 5% TCA. The centrifugates were discarded, and the precipitate, containing proteins and lipids, were extracted successively with alcohol, a mixture of alcohol and ether during which it was heated, and ether. The combined centrifugates comprised the lipid fraction. The precipitate is the protein fraction, and was freed from traces of ether by heating on a water bath. The radioactivity was measured in the tissue homogenate and separately in the lipid and protein fractions.

A. M. Kats showed that thioester does not undergo any perceptible decomposition in the body, and the radioactivity measured in the tissues shortly after the injection of thioester (S^{35}) belongs to the intact molecule of the

* The bulk of the preparation was synthesized by Z. I. Bobysheva, to whom we express our deep gratitude.

compound. For this reason we expressed the results of our experiments in μg of thioester, for which each time we measured the radioactivity of the solutions of thioester injected into the animals.

EXPERIMENTAL RESULTS

With the aim of studying the peculiarities of distribution of thioester when injected by various routes, we carried out several series of experiments. In the first series the preparation was injected intravenously in a dose of 1 mg/kg and the distribution of the thioester after different intervals of time was investigated. The results are given in Fig. 1, from which it can be seen that after 5 minutes the highest concentration of the preparation is found in the lungs. In the liver and kidneys it is considerably lower. At the end of the second hour the thioester concentration in the divisions of the brain, the kidneys and the lungs falls sharply. In the liver the content of S^{35} increases in the course of time.

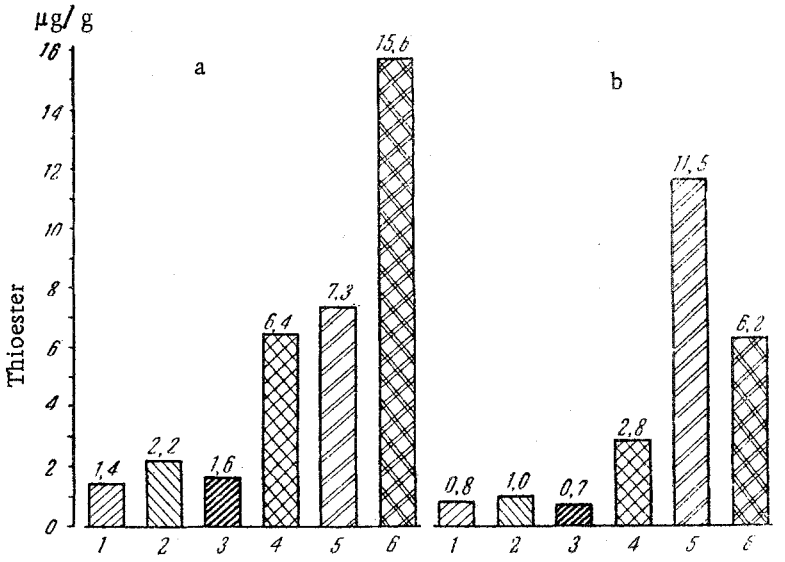


Fig. 1. Distribution of thioester in the cat after intravenous injection of the compound.
a) After 5 minutes; b) after 2 hours. 1) in the medulla; 2) in the cerebral hemispheres; 3) in the blood plasma; 4) in the kidneys; 5) in the liver; 6) in the lungs.

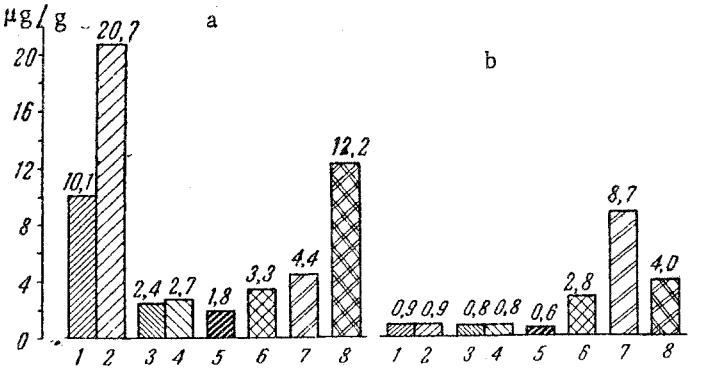


Fig. 2. Distribution of thioester in the cat after injection of the preparation into the right vertebral artery.
a) After 5 minutes; b) after 2 hours. 1) in the medulla on the left; 2) the same on the right; 3) in the left cerebral hemisphere; 4) the same on the right; 5) in the blood plasma; 6) in the kidneys; 7) in the liver; 8) in the lungs.

In the second and third series of experiments, thioester in a dose of 1 mg/kg was injected into the blood vessels supplying the brain.

The results are shown in Figs. 2 and 3. In Fig. 2 it can be seen that 5 minutes after its injection into the right vertebral artery the highest concentration of the preparation is found in the right half of the medulla. After 2 hours the thioester content of the medulla falls sharply and it becomes the same in all parts of the brain.

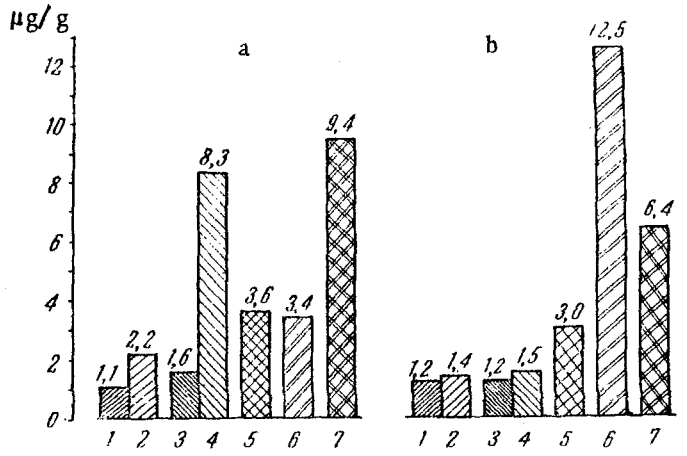


Fig. 3. Distribution of thioester in the cat after injection into the right carotid artery.
a) After 5 minutes; b) after 2 hours; 1) in the medulla on the left; 2) the same on the right; 3) in the left cerebral hemisphere; 4) the same on the right; 5) in the kidneys; 6) in the liver; 7) in the lungs.

The vertebral artery is known to enter the inferior arch of the circle of Willis and to supply mainly the brainstem. After injection into the carotid artery (Fig. 3) in the first few minutes a high concentration of thioester is created in the right cerebral hemisphere. In this case too, after 2 hours the content of the preparation in the parts of the brain becomes evened out at a low value. At the same time the content of thioester in the liver rises. In separate experiments we determined the concentration of thioester in the blood plasma. It was found to be low and practically the same after all ways of injection, nor did it change much with the course of time.

TABLE 1

Distribution of Thioester Among the Chemical Fractions of the Tissues of the Cat After Intravenous Injection of 5 mg/kg (mean results of 5 experiments; the figures expressed the radioactivity of the fractions as percentages of the total radioactivity of the homogenates)

Fractions / Organs	After 5 minutes		After 2 hours	
	protein	lipid	protein	lipid
Brain	12,14	25,71	Traces	16,63
Kidneys	7,94	21,53	»	8,14
Liver	4,95	7,21	3,59	9,50
Lungs	6,90	27,81	5,71	14,60

Thus, the study of the distribution of thioester after its injection by different routes into the body showed that shortly after injection of the preparation a high concentration is created in individual organs, but very soon a redistribution of the compound takes place. This peculiarity of the distribution of thioester may most probably

TABLE 2

Distribution of Thioester Among the Chemical Fractions of the Brain in Experiments in Vitro (incubation for 2 minutes)

Experiment No.	Concentration of thioester				
	in the homogenate (in μg/g)	in the protein fraction		in the lipid fraction	
		in μg/g	in %	in μg/g	in %
1	52,0	1,65	3,17	1,75	3,35
	304,0	11,40	3,75	1,85	0,61
	1925,0	94,50	4,91	14,00	0,73
2	32,0	1,96	6,13	1,03	3,22
	220,0	9,75	4,43	3,80	1,73
	2220,0	79,30	3,57	19,20	0,86
3	33,5	1,48	4,42	0,22	0,66
	190,0	8,21	4,32	2,17	1,14
	1870,0	72,00	3,85	15,80	0,84
4	30,8	1,65	5,36	1,05	3,41
	234,0	9,50	4,06	1,45	0,62
	2080,0	59,50	2,86	11,50	0,55
5	35,7	2,50	7,00	0,22	0,62
	242,0	10,80	4,46	1,43	0,59
	2060,0	85 70	4,16	16,60	0,81

TABLE 3

Content of Thioester (in μg/g) in the Protein Residue Before and After Dialysis

Experiment No.	Before dialysis	Time after dialysis (in hours)							%-clearance after 24 hours
		after 2	after 4	after 6	after 24	after 48	after 72	after 96	
1	61,0	53,7	44,8	49,3	18,35	14,0	—	—	69,9
2	58,6	43,7	37,6	37,8	19,45	13,7	—	—	66,8
3	50,0	40,0	40,2	40,3	—	—	—	—	—
4	106,0	—	—	—	27,1	21,9	—	—	74,4
5	100,7	—	—	—	27,3	26,0	22,0	—	72,9
6	75,5	—	—	—	31,4	18,9	16,8	10,7	58,4
7	71,7	—	—	—	23,6	17,9	14,9	10,75	67,1
8	107,0	—	—	—	33,9	—	27,2	27,2	68,3

be explained by the view that the biochemical structures of the body tissues do not possess the ability to combine firmly with the preparation and they adsorb it for only a short time.

With the aim of verifing this hypothesis we carried out a series of experiments in vivo and in vitro. In the

experiments in vivo, the preparation was injected in a dose of 5 mg/kg intravenously, the animals were decapitated after 5 minutes or 2 hours and the radioactivity of the chemical fractions of the different organs determined.

As seen from Table 1, 5 minutes after injection the proteins and lipids contain comparatively little radioactive material. After 2 hours only traces of radioactivity remain in the proteins of the brain and the kidneys, but in the lipids of these organs the content of thioester falls noticeably. In the fractions of the liver no essential changes in the concentration of thioester take place during the two hours; in this period of time the concentration of thioester in the fractions of the lungs falls.

In the experiments in vitro we incubated a brain emulsion with different concentrations of the preparation (0.02-2 mg/g) for 2 minutes, after which the tissue was fractionated and the radioactivity of the fractions determined.

It can be seen from Table 2 that with a rise in the concentration of thioester in the homogenate the radioactivity of both fractions increases, and moreover there is a particularly well expressed direct relationship between the concentrations of thioester in the proteins and the amount of the preparation added to the tissue. In the lipid fraction the same relationship was expressed, although not to such a marked degree.

We employed still higher concentrations, but in no case were we able to reach saturation limits. Thus, no stechiometric relationships exist in the interaction of thioester with the chemical structures of the brain tissue.

In order to test the firmness of combination of the drug with the proteins of the brain, experiments were performed in which a brain emulsion was incubated for 30 minutes with radioactive thioester (0.2 mg/g) and then subjected to dialysis against cold tap water (thioester is readily soluble in water). From Table 3 it can be seen that after 24 hours the bulk of the radioactivity has passed off; during the next day the process of clearance proceeds more slowly.

The study of the distribution of radioactive thioester in the animal body has shown that the preparation does not form stable compounds with the biochemical structures of the brain tissue, and that its interaction with the tissues is effected not by the formation of covalent bonds, where stechiometric relationships are essential, but evidently by means of adsorption.

SUMMARY

The author studied the peculiarities of the distribution of thioester in the animal body in different ways of its administration in experiments in vivo and in vitro. It was demonstrated that no stechiometric relationship exists in the interaction of the thioester with the chemical structure of the brain tissue.

The study of the distribution of the radioactive thioester in the animal body demonstrated that there is no stability in retention of this preparation by the biochemical structures of the brain tissue. Its interaction with the tissue substrates takes place not by the formation of the covalent connections where stechiometric relationships are imperative, but evidently as a result of adsorption.

LITERATURE CITED

[1] A. M. Kats, Biull. Eksptl. Biol. i Med. 46, 10, 61-65 (1958).*

* Original Russian pagination. See C. B. Translation.