CHANGES IN THE DISTRIBUTION AND ELIMINATION FROM ANIMALS OF THE RADIOACTIVE HYDROCHLORIDE OF THE DIETHYLAMINOETHYL THIOESTER OF DIPHENYLOXYTHIOACETIC ACID CONTAINING LABELED SULFUR

A. M. Kats

From the Laboratory of Biochemistry (Head – Prof. V. I. Rozengart) of the All-Union Research Institute of Sanitary Chemistry (Director – Dr. Med. Sci. S. N. Golikov), Leningrad

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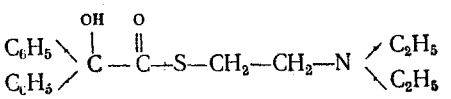
Considerable success has been achieved recently in the creation of new therapeutic preparations possessing a marked cholinolytic action. The most detailed studies have been made of spasmolytin – the diphenylacetic ester of diethylaminoethanol [3, 4, 5], the diphenylacetic ester of diethylaminoethyl-thioethanol – tiphen [1], and pentaphen – the diethylaminoethyl ester of diphenylcyclopentanecarbonic acid [2, 8].

The recently synthesized hydrochloride of the diethylaminoethyl thioester of diphenyloxythioacetic acid (thioester) is one of the members of this group of compounds:



Our aim was to study fate of this compound in animals, the character of its distribution in the body and tissues, and the route and rate of its elimination from the body.

EXPERIMENTAL METHOD AND RESULTS

For this purpose thioester containing radioactive sulfur S^{35} was synthesized.*

The preparation obtained is a white crystalline powder melting at 143-146°C and readily soluble in water and alcohol. The investigations were carried out in 4 species of animals: white rats, rabbits, cats and dogs, which were injected intramuscularly with a 1% aqueous solution of thioester – S^{35} in a dose of 5 mg/kg. The animals were killed 5, 30 and 60 minutes and 4 and 24 hours after injection of the compound, and organ tissues, plasma, urine and feces were taken for examination. The organs were thoroughly chopped up and ground in a mortar, and the homogeneous mass thus obtained was transferred to a glass plate and dried at a temperature of 65-70°C for 24 hours. The loss of weight was taken into consideration in the subsequent calculations. The dried tissues were ground to the consistency of a fine powder and sifted through a Kapronovoi sieve. Samples of 100 mg of the powder were transferred to targets to which were added 5-6 drops of alcohol and 0.5 ml of a 20% solution of KCl, which was essential in order to create a sufficiently large, dense residue on the surface of the target for the count to be made in the "thick layer." Next the targets were dried and their radioactivity measured. At the same time as the measurements were being made on the tissue sample, the radioactivity of

*The synthesis was carried out by Z. I. Bobysheva, to whom we express our gratitude.

a standard thioester solution was determined under the same conditions, thereby enabling the thioester concentration in the tissues examined to be calculated. The concentration was expressed in μg of thioester per 1 g of fresh tissue.

Content of S^{35} (Calculated as Thioester) in the Organs and Tissues of Rabbits and Cats at Various Intervals of Time after the Intramuscular Injection of the Preparation (in μg per 1 g of fresh tissue)

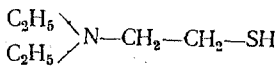
Organs and tissues	Cats	Rabbits	Cats	Rabbits	Cats	Rabbits	Cats	Rabbits	Cats
	5 minutes		30 min	60 minutes		4 hours		24 hours	
Cerebral hemispheres (grey matter)	1,61	2,80	9,70	4,60	4,71	2,64	1,50	0,81	0,50
The same (white matter)	0,57	1,07	6,41	2,43	2,88	1,88	0,89	0,73	0,46
Cerebellum	1,06	1,63	8,59	2,94	6,50	2,16	1,26	0,76	Traces
Corpora quadragemina	0,95	1,32	6,89	3,62	3,62	2,23	0,89	0,78	0,49
Optic thalamus	1,0b	1,23	6,17	3,09	0,46	2,21	0,93	0,76	0,41
Medulla oblongata	0,73	1,09	5,62	2,48	3,12	2,03	0,87	0,67	Traces
Spinal cord	0,47	0,71	2,35	1,69	1,79	1,40	0,37	0,45	»
Skin	—	0,42	2,49	—	2,23	—	2,56	—	»
Muscles	0,53	0,32	1,47	1,48	1,57	2,14	1,73	1,29	0,45
Liver	2,70	1,00	3,06	23,20	5,30	51,40	3,29	14,90	0,50
Heart	1,54	3,82	4,82	7,33	4,27	5,01	5,10	1,49	1,46
Kidneys	4,17	3,63	10,07	13,40	9,60	14,80	7,70	4,07	1,20
Lungs	16,82	22,40	32,30	21,30	21,20	18,80	11,40	2,46	0,90
Blood	2,33	2,74	3,12	6,04	3,41	7,77	1,77	1,29	0,46
Plasma	2,45	4,07	5,66	7,57	5,00	9,27	2,41	1,64	0,90
Urine	0,16	0,43	29,07	23,40	40,42	48,00	124,6	62,10	31,40

*Blood, plasma and urine in μg per 1 ml.

The character of the distribution of sulfur was found to be of the same type in all the animal species investigated. In content of S^{35} found in them, the lungs occupied first place, whereas the radioactive sulfur content of the central nervous system was comparatively small. This difference was particularly pronounced at short intervals of time after injection of the drug (see Table). A clearly marked relationship was observed between the quantity of S^{35} found and the time after injection of the preparation. Thus in the majority of organs the S^{35} content rose in the first 30 minutes and then began to fall progressively. The quantity of S^{35} in the urine rose considerably over a period of 4 hours. At the end of the first 24 hours the content of radioactive sulfur in the majority of organs became very low. Such a rapid elimination of radioactive sulfur from the organs led us to put forward the hypothesis that thioester does not take part in the formation of any stable compound with the biochemical tissue substrates, and rapidly leaves the body.

The question naturally arises, what is the nature of the radioactive sulfur found in the tissues after injection of thioester. Bernheim [6], Kisch and his co-workers [9] and Pulver [10] point out that the first step in the conversion of drugs with the structure of complex esters is their decomposition at the ester bond. Since thioester belongs to this particular class of compound, it might be suggested that it will be broken down in the body into its component alcohol and acid. If so, then it is possible that the radioactive sulfur found in the organs and tissues belongs not to the intact molecule of the preparation but to the product of its hydrolysis.

The most likely product of the hydrolysis of thioester to contain sulfur is diethylaminoethanethiol. For the purpose of establishing to what compound the radioactive sulfur detected in our experiments belongs, a series of investigations was carried out in which rabbits were injected with the product of hydrolysis of thioester labeled with sulfur – diethylaminoethanethiol (thioalcohol):



Diethylaminoethanethiol

Thioalcohol was injected in an equimolecular dose, and determination of the radioactivity was carried out at the same intervals of time as after injection of thioester.

As a result it was established that the character of distribution of sulfur differed after injection of thioester from that after injection of its product of hydrolysis – thioalcohol. This difference was particularly noted at early stages after injection (Fig. 1). The maximum quantity of S^{35} after injection of thioalcohol was found in the kidneys, whereas after injection of thioester of greater quantity was found in the lungs. In the divisions of the central nervous system the absolute quantity of sulfur after injection of thioalcohol was significantly greater than after injection of thioester. The findings obtained in this series lead us to consider that at short intervals after injection the S^{35} content reflects the pattern of distribution of the intact thioester molecule among the organs and tissues of the animal and that the preparation is present in the body mainly in an unsplit form.

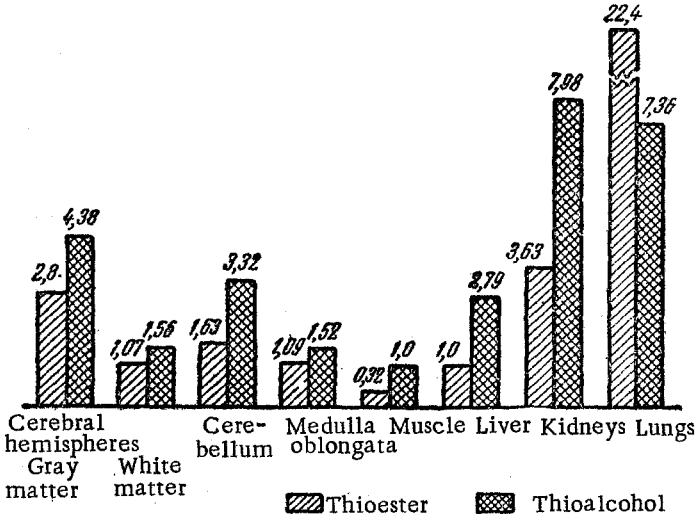


Fig. 1. Distribution of S^{35} in the organs of rabbits 5 minutes after the intramuscular injection of thioester and thioalcohol.

In the light of these findings it was interesting to study the ability of the tissues to carry out enzymic decomposition of thioester in order to ascertain whether it is possible in general for the enzymic hydrolysis of this compound to take place in the body.

For this purpose a series of experiments was set up in vitro, using the chemical method of determination of complex ethers suggested by Hestrin [7] which we modified. The experiments were performed as follows. The liver, brain, kidneys and lungs were cut up finely with scissors on ice, samples weighing 500 mg of each tissue were transferred to two tubes, 1.5 ml of a phosphate buffer was added to each and half the tubes were placed for 5 minutes in a boiling water bath. After this, to all the tubes were added 1 ml of a 1% solution of thioester, and they were then placed for a definite time in the incubator at a temperature of 37°C. At the end of incubation the quantity of residual unsplit thioester in all the tubes was determined.

Further confirmation of the idea that the preparation is in practice not split up in the body was provided by biological test experiments, based on the fact that a mydriatic effect is a property which is peculiar to the intact thioester molecule. The method of performance of these experiments was as follows. A rabbit was injected intramuscularly with thioester solution, and then after a definite time its urine was extracted by puncture of the bladder, and injected into the eye of another rabbit. In this way a marked mydriatic action was

observed. From a comparison of all these findings it could be concluded with sufficient confidence that thioester undergoes practically no significant degree of decomposition in the body and is excreted in an unchanged form.

It was established that under these experimental conditions enzymic hydrolysis of the preparation does not take place. Incubation even for four hours did not result in any perceptible diminution in the quantity of the preparation added to the tissues. In an experiment using highly purified liver esterase, the whole quantity of preparation added was also detected in its entirety.

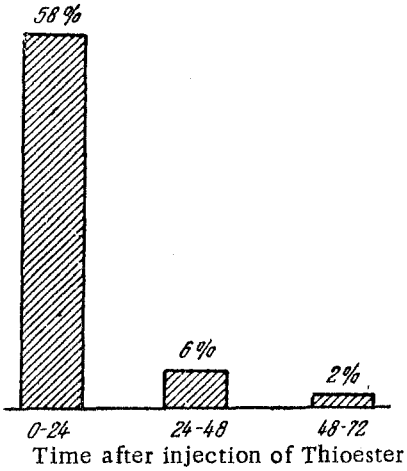


Fig. 2. Excretion of S^{35} by rats after intramuscular injection of thioester.

Experiments in which, besides the distribution, the content of the preparation in the urine was studied, showed that it is present there 5 minutes after injection. The rate of excretion of thioester was studied in special experiments on dogs with ureters transplanted under the skin of the abdominal wall. It was shown that the preparation leaves the body very rapidly, mainly in the urine. The role of the gastrointestinal tract in the process of excretion of thioester was found to be very insignificant.

In order to make a closer study of the process of excretion of the preparation, experiments were set up on rats. In 4 series of experiments the excretion of thioester was investigated 12, 24, 48 and 72 hours after the moment of injection of a 1% aqueous solution of the preparation in a dose of 10 mg/kg (Fig. 2).

During the first 24 hours the urine of the rats was found to contain on the average 58% of the dose injected, during the second 24 hours – 6% and during the third 24 hours – 2% altogether. It was shown that the greater part of the preparation (55%) was excreted by the rats in the first 12 hours. Later still the excretion of the preparation became very slight.

As a result of the work which has been carried out a number of the distinctive features of the distribution of thioester at different periods after its injection have been made clear. It has been established that the preparation rapidly leaves the body, mainly in the urine. These phenomena may be associated with the transient therapeutic action of thioester. The results of the experiments also give grounds for the belief that a considerable part of the preparation is excreted from the body in an unchanged form. One of the future tasks in the field of study of the fate of cholinolytic substances in the body must be to determine the distribution and the rate of excretion of the different preparations of this type with a view to establishing the relationship between the changes in the distribution of the preparations in the body and their chemical structure and physicochemical properties.

SUMMARY

Chlorhydrate of diethylaminoethyl S-ester of diphenyloxythioacetic acid (thioester) demonstrated the specific peculiarities of distribution in intramuscular injection to dogs, cats, rabbits and rats. Its maximal quantities are revealed in the lungs in a short time (up to 1 hour) after the administration of the thioether. The product of hydrolysis of the thioester, the thioalcohol, differs from the thioester by its distribution. The maximal quantitity of thioalcohol is revealed in the kidneys at the same time interval.

Evidently, the thioester does not undergo any significant fermentative disintegration in the animal body and is rapidly excreted in the urine, mostly in unchanged condition. The role of the gastrointestinal tract in the process of thioester excretion is rather insignificant.

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