THE METABOLISM OF HYDROGEN SULFIDE (H_2S^{35}) INJECTED SUBCUTANEOUSLY

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(Received June 26, 1956. Presented by S. V. Anichkov, Regular Member of the Academy of Medical Sciences of the USSR)

The metabolism of exogenous hydrogen sulfide in an organism has been studied by a number of authors [3, 4, 5, 6, 7, 9, 10]. It has been established that hydrogen sulfide is rapidly oxidized and is transformed on the whole into pharmacologically inactive sulfates. Insufficient light has been shed on the question of the possible inclusion of exogenous hydrogen sulfide or its oxidation products in the proteins of organs and tissues. Tarver and Schmidt [10] noted that S^{35}-sulfates do not enter into the construction of the sulfur-containing amino acids. Smith, Anderson and Odell [9] have shown that intravenously injected S^{35}-sulfates are combined in the blood with a compound possessing electrophoretic mobility similar to the mobility of α_1-globulin. The authors consider that this compound is a protein with a sulfur-containing carbohydrate prosthetic group.

The aim of our work was to study the metabolism of hydrogen sulfide containing S^{35} after subcutaneous injection of Na_2S^{35}.

The direct injection of large amounts of sulfide into the blood is limited by its high toxicity. Significantly greater amounts of sulfide can be injected subcutaneously than can be injected intravenously. According to V. A. Tikhonravov and A. N. Iskova [7] lethal doses of hydrogen sulfide in the case of subcutaneous injection are 10-35 times larger than in the case of intravenous injection. This is explained by the fact that the hydrogen sulfide, gradually permeating from the subcutaneous tissue into the blood, has time to be oxidized and does not reach toxic concentrations in the blood.

EXPERIMENTAL METHODS

Experiments were conducted on 34 male white rats weighing from 160 to 230 g. Na_2S^{35} was injected subcutaneously in the abdominal region in a single dose corresponding to 0.2-0.5 mg of H_2S^{35} (from 6×10^5 to 1.7×10^6 impulses/min). In some of the experiments Na_2S^{35} was injected daily for a period of 4-5 days (in all from 3×10^5 to 11×10^6 impulses/min were injected during an experiment). Rats were decapitated 1, 2, and 6 days after injection of Na_2S^{35}. A determination was made of the H_2S^{35} in the blood, organs and urine. The distribution of S^{35} was studied in dry preparations of the organs, blood and urine as well as in the proteins of the organs. In order to calculate specific activity the total sulfur content of the organs was determined. The overall amount of sulfur and inorganic sulfates in the urine was determined.

For the H_2S determination blood or ground tissue was acidified, and the H_2S forced out with a stream of nitrogen into two receiving vessels containing 2 ml of 0.1 N NaOH. The determination of H_2S in the alkaline solution was performed according to the Karo-Fischer method. The details of the determinations were described earlier by us and by V. A. Tikhonravov [1]. Aside from the photometric determination of H_2S an impulse count

was made of the receptacle. The sensitivity of the chemical method under the conditions of the technique employed amounted to 0.04 γ H_2S per ml, while that of the radiometric method was 0.002-0.0004 γ H_2S^{35} per ml.

The determinations of the urine sulfur fractions were performed by the gravimetric method. The total sulfur content of the organs was determined by the gravimetric method with prior hydrolysis by boiling for 10-12 hours in 10% NaOH. Barium sulfate was collected by centrifuging in special conical test tubes. The samples for the count were prepared in a kind of suspension (2.5 mg $BaSO_4$ and 0.05 ml of 70% alcohol), which was distributed uniformly over a target. The preparations were dried slightly at 50-60°.

Natural preparations were prepared for a count from organs finely sliced and ground in a mortar (0.1-0.3 g). The count was made after slight drying. The protein preparations were prepared by the ordinary method of precipitation with trichloroacetic acid. They were washed in a liquid rinse prior to the disappearance of radioactivity. The dried preparations were triturated, sifted and placed in 50 mg amounts in tin foil targets (of the size of a 10 kopeck coin). The count of the activity of all the specimens was carried out in a lead booth by means of an end counter. The duration of the count was determined by the magnitude of the activity of the specimen (from 5 to 15 minutes). The radioactivity counts were made several times during the working day. The sodium sulfide containing radiosulfur included an admixture of sulfates. Therefore, in order to standardize it and purify it of contaminants, before each experiment the solution was acidified and H_2S was forced out into 0.1 N NaOH. Such freshly prepared Na_2S^{35} solutions were used in all of the experiments.

RESULTS OF THE EXPERIMENTS

The hydrogen sulfide content of the blood was determined in 22 rats. H_2S was not detected in a single case, neither by the chemical nor the radiometric method. Analogous results were obtained in the examinations of the lungs and urine. Hydrogen sulfide was found in the liver in 5 cases by the chemical method, but was not detected in a single case by the radiometric method.

The hydrogen sulfide detected in the liver we consider to be endogenous, since hydrogen sulfide was not infrequently found in the liver of normal rats without its injection from without. Had the subcutaneously injected hydrogen sulfide been present in the liver, it would have been detected by the radiometric method.

TABLE 1

Elimination of S^{35} among Rats after Subcutaneous Injection of NA_2S^{35}

Time after injection in days	Experiment 1				Experiment 2			
	Na_2S^{35} was injected subcutaneously							
	220 γ (744 × 10^3 impulses/min)				182 γ (602 × 10^3 impulses/min)			
	Total sulfur, imp/min/ml	Radioactivity in %		% of the injected number of imp./min	Total sulfur, imp/min/ml	Radioactivity in %		% of the injected number of imp/min
		inorganic sulfates	neutral sulfur			inorganic sulfates	neutral sulfur	
1	71 786	97.8	2.2	55	—	—	—	—
2	10 681	92.3	7.7	5.5	2916	87.1	12.9	3.1
3	—	—	—	—	336	65.2	34.8	0.84
4	1 059	—	—	0.6	108	100	—	0.21
5	1 835	54.4	45.6	1.5	218	63.7	36.3	0.32
6	—	—	—	—	85	70	30	0.12

The absence of H_2S^{35} after large amounts of Na_2S^{35} have been injected into an organism can be explained in accordance with facts well-known in the literature [4, 5, 6, 7] of its oxidation and, to a lesser extent, its combination with organic compounds. From the subcutaneous tissue the H_2S^{35} slowly permeates into the blood where it is oxidized. From the blood the products of the transformation of H_2S pass into the tissues and are eliminated in the urine. In corroboration we adduce the results of an examination performed on 2 rats over a period of 6 days after a single injection of Na_2S^{35} (Table 1).

As is apparent from Table 1, the greatest amount of the injected S^{35} is eliminated the first day. Inorganic sulfates make up the greatet part of the total sulfur. Elimination of S^{35} falls off sharply during the subsequent days. Prolonged elimination of small amounts of S^{35} apparently results from the distribution of S^{35} within the organism among other sulfur-containing metabolic products, together with which the S^{35} is gradually eliminated. It is possible, however, that part of the S^{35} is included in organic compounds and is eliminated from the organism only with their disintegration. In order to shed light on this problem a study was made of the distribution of S^{35} (per cent specific activity of S^{35} [2]) in the organs of the rat 1 day after (Experiment 1) and 6 days after (Experiment 2) a single injection of Na_2S^{35}.

TABLE 2

Specific Activity of S^{35} in the Organs of Rats

Organs	Experiment 1			Experiment 2		
	Na_2S^{35} was injected subcutaneously					
	168 γ (871 × 10^3 imp/min)			520 γ (968 × 10^3 imp/min)		
	sulfur in mg/g of tissue	imp/min per g of tissue	Spec. act. × 10^{-2}	sulfur in mg/g of tissue	imp/min per g of tissue	Specific activity, × 10^{-2}
Lungs	1.0	675	7.7	1.3	244	2.7
Liver	1.4	1 007	8.2	1.6	144	0.9
Kidneys	0.5	1 363	32.2	1.9	363	1.9
Spleen	1.7	2 742	19.5	1.4	294	2.2
Brain	0.6	418	7.7	0.7	197	2.7
Heart	1.8	514	3.2	2.2	132	0.6
Skeletal muscles	1.1	89	0.9	1.8	107	0.6

It is apparent from Table 2 that the greatest specific activity 1 day after injection of Na_2S^{35} is observed in the kidneys, then in the spleen and liver. This is explained by the removal by the kidneys of the products of the metabolism of H_2S. The least activity was detected in the skeletal muscles. Six days after injection of the sulfide the specific activity falls off considerably. The greatest activity is detected in the lungs and brain, the least in the muscles. Apparently the residual activity is due to a comparatively more stable connection of S^{35} with the organs. One may suppose that part of the S^{35} is included in the proteins of the organs.

TABLE 3

S^{35} Content in Protein Preparations of the Organs

Organs	Na_2S^{35} was injected subcutaneously		
	46 γ (3.5×10^6 imp/min)	72 γ (8.3×10^6 imp/min)	495 γ (11.1×10^6 imp/min)
	S^{35} content (no. of impulses/min in the heavy layer)		
Lungs	10	39	94
Liver	0	15	29
Kidneys	8	110	185
Spleen	4	41	76
Heart	2	14	33
Brain	3	14	74
Skeletal muscles	0	8	29

In order to shed light on this question a determination was made of the content of S^{35} in the protein preparations of organs. With the aim of increasing the amount of S^{35} injected into the organisms Na_2S^{35} was subcutaneously injected daily into rats for a period of 3-5 days. The determinations were carried out 24 hours after the last injection of the sulfide (Table 3).

It is apparent from Table 3 that, when the amount of sodium sulfide injected is increased, the S^{35} content in the protein preparations of the organs increases. The radioactivity connected with the proteins of the organs, however, proved to be very slight. We do not have data available on the nature of the connection of S^{35} with the tissue proteins. Apparently the presence of S^{35} in protein preparations of the organs cannot be explained by the simple adsorption of sulfates. In order to shed light on this question we treated the protein preparations of the organs for 2-24 hours at room temperature with a 0.001-0.003 M solution of Na_2SO_4 and examined the centrifugates. S^{35} was not detected in the centrifugates.

Our research confirms the fact that in the processes of the transformation of hydrogen sulfide within an organism the utilization of sulfur in the construction of organic compounds is of minor importance in comparison with the fundamental process, that of removal by the kidneys of the oxidation products of H_2S.

SUMMARY

White rats were subcutaneously injected with Na_2S^{35}. The content of hydrogen sulfide in the blood, organs and urine was determined chemically and radiometrically on the 1st, 2nd, and 6th day. The distribution of S^{35} was studied in dried preparations of organs, blood, urine and in the proteins of the organs.

No hydrogen sulfide was detected in the blood, urine and lungs. The greater part of S^{35} was removed from the organism with the urine in the first 24 ohours. The utilization of sulfur in the formation of organic compounds is of secondary importance in comparison with the removal of oxidation products of H_2S by the kidneys.

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