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Studies on Mustard Gas ($\beta\beta'$ -Dichlorodiethyl Sulphide) and some Related Compounds

5. THE FATE OF INJECTED MUSTARD GAS (CONTAINING RADIOACTIVE SULPHUR) IN THE ANIMAL BODY†

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Young (1941) showed that when H^* (mustard gas containing radioactive sulphur) is applied to the tail of a rat, some of the S^* (radioactive S) derived from the H^* passes from the site of application, and is later found in the liver, kidneys and urine of the animal, and Salter (1942) found considerable amounts of S^* in the bone marrow of rats 3 hr. after H^* had been applied to the skin. Cameron (1941) has described the systemic damage occurring in H -treated animals, and Needham, Cohen & Barrett (1943), in investigations on the systemic effects of H , have drawn attention to the fact that there is inhibition of glycolysis in bone marrow, and that there is an appreciable delay in the onset of this inhibition, parallel with the delay in the appearance of histological damage. They also showed that, in contrast, the action of H on the enzymic systems of bone marrow *in vitro* is almost immediate. One possible explanation for the delay, discussed very tentatively by Needham *et al.*, is that H or some derivative of it might be stored in the fat of the bone marrow or elsewhere in the body and released slowly over a long period. In the hope of throwing some light on these problems, we have carried out investigations involving the injection of H^* into rabbits and the determination of the distribution of S^* in the blood and other tissues of the animals at various intervals (up to 12 hr.) after the injections. In other experiments the excretion of S^* in the urine

and the bile of rabbits injected with H^* has been investigated.

In these experiments, two types of determination of the S^* content of a tissue have been made, one involving a determination of the total S^* and the other the 'fixed' or firmly combined S^* . For the total S^* determinations tissues were dried *in vacuo* but were not extracted with any solvent; these results give a measure of the combined and uncombined H^* plus any other S^* -containing substance present in the tissue at the time that the rabbit was killed. The 'fixed' S^* determinations were made on the minced tissues after they had been exhaustively extracted with various solvents, to remove loosely attached H^* and related compounds, and then dried; it is believed that these values give a measure of the amount of H^* which has combined with the proteins and perhaps other constituents of the tissues. The use of the term 'fixed' is not intended to imply that the attached S^* will necessarily remain fixed in the tissues *in vivo*, and, indeed, evidence is presented to the contrary, for in some of the experiments the results suggest that there is, in the intact animal, fairly rapid removal of this 'fixed' S^* from the tissues. In the majority of cases where determinations were made of both total S^* and 'fixed' S^* , the two values were of the same order when both results were given per g. of material dried over CaCl_2 *in vacuo*; occasionally, however, the 'fixed' S^* value was appreciably higher than the total S^* , presumably due to the extraction by the solvents of fatty substances containing little or no S^* .

† The investigations described in this paper were the subject of reports to the Ministry of Supply in 1943-5.

METHODS

Injections. The H^* , prepared as described previously (Bourns, Francis & Wormall, 1946a), was dissolved in triacetin to give a 5% solution. This solution was injected into the marginal ear vein of a rabbit in amounts equivalent to 5 mg. of $H^*/\text{kg.}$ body wt.

Collection of the blood samples. Blood was taken from the ear not used for the injection and was collected in tubes containing a 0.1% heparin solution in 0.9% NaCl (about 5% of the volume of blood to be collected). The heparin was part of a sample kindly presented to one of us (A. W.) some years ago by Dr J. E. Jorpes.

Treatment of the blood samples. 5 ml. samples were centrifuged, the plasma separated and the cells washed 3 times with about 3 ml. of 0.9% NaCl each time, the washings being added to the plasma. The cell and plasma fractions were incinerated without further treatment.

Treatment of the tissues. The injected rabbit was killed and the tissues immediately excised and cut into small pieces; in some of the later experiments they were minced in a Latapie mincer. The stomach and duodenal walls were washed with tap water to remove the adhering contents before the tissue was dried, but the mucosa, muscularis and serosa were retained in the sample to be analyzed. The heart was cut open and washed to remove the blood from the chambers.

Total S^* determinations were made on the products obtained by drying a weighed amount of the minced wet tissue (usually about 2–3 g.) *in vacuo* over CaCl_2 to constant weight. The results can thus be expressed as $\mu\text{g.}$ of $S^*/\text{g.}$ of wet or dry tissue.

For 'fixed' S^* determinations, the minced tissue was kept under acetone for some time, ground to a fine powder with liquid air, then Soxhlet-extracted (for four 3 hr. periods with ether and acetone in rotation) and finally dried *in vacuo* over CaCl_2 to constant weight.

S^* determinations. The solution or solid was incinerated with HNO_3 , NaClO_3 and Cu wire, and the S^* precipitated and determined as BaSO_4^* as described previously (Bourns *et al.* 1946a), using the Geiger-Müller counter system described by Banks (1946).

Total N (Kjeldahl). In many cases total-N determinations were made on the samples used for S^* determinations in order that an approximate measure of the maximum protein content of the tissue might be obtained.

RESULTS

Distribution of S^ in the tissues after the injection of H^* into rabbits*

Five rabbits were intravenously injected with H^* as described above, and two (A and B) were killed 1 hr. and the others (C, D and E), 2½, 4 and 12 hr. respectively after the injection. In all cases, S^* determinations were made on the liver, kidney, lung and bone marrow (tibial and femoral), and with some rabbits a number of other tissues were also examined. Tables 1–3 and Fig. 1 give the results of this experiment, which was designed to determine (a) how quickly the H^* (or derivatives of H^*) penetrated into the various tissues and how long it

remained there, and (b) whether there was preferential retention of the S^* in any tissue.

Table 1. 'Total' S^* in rabbit tissues at intervals after the intravenous injection of H^* (5 mg. of $H^*/\text{kg.}$ of body weight)

	Rabbit killed after (hr.)	Dried tissue		Wet tissue S^* ($\mu\text{g./g.}$)
		N (%)	S^* ($\mu\text{g./g.}$)	
Kidney	1 (A)	11.1	13.2	3.5
	1 (B)	12.0	20	4.6
	2½ (C)	11.2	13	3.0
	4 (D)	—	—	—
	12 (E)	11.1	3.4	0.83
Liver	1 (A)	10.9	4.8	1.4
	1 (B)	—	5.8	1.8
	2½ (C)	10.7	2.6	0.48
	4 (D)	11.2	2.6	—
	12 (E)	11.3	1.6	0.47
Lung	1 (A)	11.9	6.3	1.3
	1 (B)	12.2	8.4	1.9
	2½ (C)	10.8	11	2.1
	4 (D)	8.8	18	—
	12 (E)	12.5	3.2	0.61
Bone marrow†	1 (A)	—	—	—
	1 (B)	—	0.7	0.5
	2½ (C)	1.53	0.02	0.01
	4 (D)	1.04	0.3	—
	12 (E)	1.04	0.08	0.06

A, B, C, D and E refer to the rabbits (each about 2.5 kg. in wt.) used in this experiment.

† Owing to the relatively low water-content of this tissue, there was less difference between the S^* contents of the wet and dried tissues in the case of bone marrow than with the other tissues examined.

Table 2. 'Total' S^* in other rabbit tissues after the intravenous injection of H^* (5 mg. of $H^*/\text{kg.}$ of body weight)

	Rabbit killed after (hr.)	Dried tissue		Wet tissue S^* ($\mu\text{g./g.}$)
		N (%)	S^* ($\mu\text{g./g.}$)	
Perfused kidney	2½ (C)	10.1	8	1.4
Spleen	4 (D)	10.5	7	—
Spleen	12 (E)	12.2	0.88	0.22
Stomach wall†	1 (B)	11.8	2.1	0.5
Duodenal wall†	1 (B)	12.2	4.7	0.86
Brain	1 (B)	8.4	1.1	0.3
Heart	1 (A)	9.3	3.1	0.7
Muscle†	1 (A)	12.4	2.1	0.5
Skin	1 (A)	—	—	0.2
Thyroid	1 (A)	—	—	Less than 0.5

† The stomach and duodenum were washed to remove their contents, but the mucosa, muscularis and serosa were included in the tissue examined.

‡ Representative portion of entire minced skeletal muscle. A, B, C, D and E refer to the rabbits used in this experiment (cf. Table 1).

Table 3. 'Fixed' S* in rabbit tissues at intervals after the intravenous injection of H* (5 mg. of H*/kg. of body weight)

	N (%)				S* ($\mu\text{g./g. dry tissue}$)			
	Rabbit killed after (hr.)				Rabbit killed after (hr.)			
	1 (A)	1 (B)	2½ (C)	4 (D)	1 (A)	1 (B)	2½ (C)	4 (D)
Kidney	12.3	12.4	—	12.1	21	16	—	10.5†
Liver	12.4	11.6	11.7	12.4	4.8	7.5	3.8‡	5.5
Lung	12.7	12.4	—	12.4	6.8	3.5	—	14
Muscle§	13.7	—	—	—	0.85	—	—	—
Brain	—	11.8	—	—	—	3	—	—
Stomach	—	12.8	—	—	—	0.6	—	—
wall	—	—	—	—	—	—	—	—
Duodenal	—	12.7	—	—	—	6.0	—	—
wall	—	—	—	—	—	—	—	—

A, B, C and D refer to the rabbits used in this experiment (cf. Table 1).

† This kidney was perfused with Ringer solution via the blood vessels for about 10 min.

‡ This material was powdered under cold acetone, but was not Soxhlet-extracted.

§ and || see footnotes to Table 2.

Fig. 1. Total S* in tissues of rabbits after the injection of 5 mg. of H*/kg. of body wt.

S* content of the blood of rabbits after the injection of H*

Experiments have been carried out to determine the distribution of S* between the plasma and the cells of the blood at intervals after the injection of H*.

Rabbit F was injected with 5 mg. of H*/kg. of body wt., and blood samples (5–6 ml.) were taken at intervals up to 12 hr. Total S* determinations were made on the separated plasma (plus cell washings) and cells from 5.0 ml. blood samples. The results of this experiment are shown in Fig. 2.

The graphs in Fig. 2 show that throughout the whole period of the experiment about two-thirds of the S* in the blood was present in the plasma, the actual percentage being a little higher during the first hour than it was thereafter. The blood cell S* remained fairly constant for the first 2 hr. and then fell slightly, but the plasma S* fell considerably during the period 1–4 hr. after the injection, presumably as a result of gradual diffusion of H*, H*-

hydrolysis products or other diffusible H*-compounds into the tissue spaces, urine, etc.

A point of interest arising from these results is that the S* content of the blood during the early stages of the experiment, i.e. 20 min. and 1 hr. after the injection, was only about one-tenth of that which would be expected if the H* were evenly distributed throughout the vascular system and retained in the blood. This observation supports the view that an appreciable part of the injected H* rapidly diffuses into the tissue spaces, or is excreted in the urine or the bile, as is shown in experiments described below.

Fig. 2. Total S* in the blood cells and plasma of a rabbit after the injection of 5 mg. of H*/kg. of body wt.

In connexion with these determinations on blood, a control *in vitro* experiment has been carried out to determine (a) the 'recovery' of S* when determinations are made on H*-blood mixtures, and (b) the distribution of S* between the plasma and cells in these mixtures. 6 mg. of H* (5% solution in triacetin) were added to 90 ml. of heparinized rabbit blood and the mixture was stirred at 37° for 12 hr. Samples were taken at intervals and total S*-determinations were made on the separated washed cells and plasma (plus cell washings). The results showed a low recovery of the added S*, the values being consistently about 20% below the theoretical values; this was not unexpected, for it has been noted previously (Bournsnel *et al.* 1946a) that although the method used for S and S* determinations gives theoretical values with H, HO, HO₂ and with HO₂-amino-acid derivatives, the recovery with hydrolyzed H is invariably low. In this experiment the cell S*/plasma S* ratio was 1.6 in the samples taken 20 min. after the addition of the H*, and 0.6 after 1 hr., at which level it remained fairly constant for the rest of the experiment. This suggests an initial combination of much of the H* with the red cells and a later dissociation of this complex. [Cogan, Kinsey & Grant (1942) have noted a rapid association

of H with red cells *in vitro*.] In the *in vivo* experiment described above (rabbit F) the change was different, for the ratio increased slowly from 0.38 at 20 min. to 0.63 at 12 hr. after the injection of the H^* .

The excretion of S^ in the bile following injection of H^* into rabbits*

Evidence was obtained in an experiment described elsewhere (Boursnell *et al.* 1946*b*) that the bile contains considerable quantities of S^* after the intravenous injection of HO^* into a rabbit, and it was therefore decided to study the excretion of S^* in the bile of a cannulated rabbit after the injection of H^* . The contents of the alimentary tract of the injected rabbit were also examined to determine whether or not S^* is excreted there even when no bile enters the tract.

Experimental details. A female rabbit (J, 2.7 kg.) was anaesthetized by an intravenous injection of urethane (1.5 g./kg. of body wt.). A cannula was inserted into the bile duct about 1 cm. from the entrance into the duodenum, the duct being ligated between the cannula and the duodenum. The gall bladder was tightly ligatured at the neck and emptied with a syringe.

30 min. after the completion of the operation, the cannula was emptied, and a solution of H^* in triacetin (5 mg. of H^* /kg. of body wt.) was intravenously injected into the rabbit; thereafter the cannula was emptied at frequent intervals, and total-N and total- S^* determinations were carried out on the weighed samples of bile obtained. All the total-N values were of the same order of magnitude (varying between 0.074 and 0.094%).

The rabbit was kept warm with lamps and cloths, and intravenous injections of warm saline (10 ml.) were made at hourly intervals. The rectal temperature 15 min. after the injection of H^* was 37.5° and it rose slowly to 39.0° at 3½ hr., when the rabbit died suddenly. Immediately after the death of the animal, the alimentary canal was tied off in sections and removed. The contents of each section were washed out into a separate vessel, and treated as follows for S^* determinations.

(a) The whole of the stomach contents was evaporated after the addition of a little NaOH (to prevent loss of H_2S^*), and re-evaporated with conc. HNO_3 . It was then boiled with a mixture of 2 vol. of conc. HNO_3 and 1 vol. of 60% $HClO_4$ until a clear solution was obtained. This solution was diluted to 250 ml. and sample portions were precipitated with $BaCl_2$.

(b) The contents of the duodenum, and those of the middle and caudal parts of the small intestine, were separately made alkaline, evaporated and then re-evaporated with conc. HNO_3 . The products were transferred to incineration tubes and treated in the usual manner for S^* determinations.

(c) The contents of the caecum and large intestine were treated as in (a), but the material was lost by an explosion caused by the mixture with $HClO_4$ being allowed to boil almost dry.

S^* determinations. These were carried out in the usual manner (Boursnell *et al.* 1946*a*) with the following slight modifications. A small amount of Na_2SO_4 solution (equivalent to 15 mg. of $BaSO_4$) was added to each bile sample

before the $BaSO_4^*$ was precipitated, to ensure a suitable amount of $BaSO_4^*$ for radioactivity measurements. Where the radioactivity of the samples was expected to be low (e.g. with stomach contents), amounts of $BaSO_4^*$ rather larger than usual were spread (up to 50 mg.) and the appropriate 'self-absorption' corrections applied as described by Banks (1946).

The excretion of S^* in the bile of the injected rabbit is shown in Fig. 3. Within the first 10 min. a significant amount of S^* (7.3 μ g.) was excreted, and during the 3½ hr. that the animal survived a total amount of 200 μ g. was found in the bile, i.e. a little over 7% of the S^* in the H^* injected. Even in the last specimen of bile obtained, the concentration of S^* was still appreciable (5.4 μ g./g. of bile), but the rate of flow of the bile had decreased very considerably. It appears probable, therefore, that even more S^* would have been excreted via this channel had the flow of bile been maintained.

The amount of S^* in the contents of the alimentary tract was relatively very small, viz. stomach (15 μ g.); duodenum (1.5 μ g.); small intestine, middle portion (13 μ g.); small intestine, caudal portion (3.5 μ g.).

Fig. 3. The excretion of S^* in liver bile after the injection of H^* into a rabbit (13.5 mg. of H^* , i.e. approx. 2700 μ g. of S^*). A, total amount secreted (μ g. of S^*); B, concentration (μ g. of S^* /g. of bile).

The excretion of S^ in the urine and faeces following the injection of H^* into rabbits*

In order to obtain as complete a picture as possible of the fate of injected H^* , experiments have been carried out to determine the S^* content of the urine of rabbits at intervals after the injection of H^* .

Three rabbits were anaesthetized, and glass canulae, made in the form of thistle funnels, were tied into their bladders in such a way as to permit the minimum of dead-space without obstructing the openings of the ureters. Anaesthesia was first produced by an intravenous injection of 14 ml. of 20% urethane, and maintained by occasional small quantities of ether. The animals were kept warm by cloths and lamps. As the volume of urine secreted by rabbits is small, particularly by the anaesthetized animal, the flow was stimulated by the intravenous injection of 0.9% NaCl at intervals. The times of these injections are indicated by arrows in Fig. 4.

rose from 37.2 to 37.9° during the experiment. The rabbit was killed 4 hr. after the injection.

Rabbit L. Female, 2.20 kg.: 100 ml. of water given by stomach tube beforehand. The rectal temperature fell from 38.0 to 37.0°. The animal was hyperventilating throughout the experiment. Death occurred 4½ hr. after the injection of the H^* .

Rabbit M. Male, 2.50 kg.: rectal temperature fell from 37.8 to 35.2°. The animal was killed 7 hr. after the injection.

The whole of the pinna of rabbit K used for the injection of the H^* was analyzed in sections for S^* content, in order to determine whether any appreciable amount of S^* remained at or near the site of

Fig. 4. Urinary excretion of S^* after the injection of H^* into rabbits [5 mg. of H^* (containing approx. 1000 μ g. of S^*)/kg. of body wt.]. —○— urine secreted (g./kg.); —×— excretion of S^* (μ g./kg.); ↑ injection of 10 ml. of warm saline; * injection of 15 ml. of warm saline.

A 5% triacetin solution of H^* (5 mg./kg. of body wt.) was injected intravenously a few minutes after the completion of the operation, and the urine samples were collected at intervals in weighed incineration tubes; these samples were treated with HNO_3 , etc., and the sulphate precipitated as $BaSO_4^*$ after the addition of a known amount of Na_2SO_4 (added to give a suitable amount of $BaSO_4$ for spreading).

Rabbit K. Female, 2.80 kg.: 100 ml. of water administered by stomach tube beforehand. The rectal temperature

the injection. With another animal (M), the S^* in the contents of the alimentary tract was determined, for comparison with the results obtained with rabbit (J) whose bile-duct had been cannulated; the collection and treatment of the contents followed closely those described for the experiment with rabbit J.

The amounts of S^* excreted in the urine of rabbits K, L and M are given in Table 4, and the rates of excretion of urine and of urinary S^* are shown in Fig. 4. For purposes of comparison, S^* -determinations were made on the urine taken from the bladder

of another rabbit (A, of 1.85 kg.) immediately after the animal had been killed (1 hr. after the injection of 5 mg. of H^* /kg. of body wt.). This rabbit, which was not anaesthetized or subjected to surgical treatment, was found to have 121 μ g. of S^* in the urine (c. 3 ml.) in its bladder. No S^* was detectable in the sample of urine (42 ml.) passed by this animal 6 min. after the injection.

Table 4. *Urinary excretion of S^* by rabbits after the intravenous injection of 5 mg. of H^* /kg. of body weight*

Time interval (min.)	Rabbit K		Rabbit L		Rabbit M	
	Urine (g.)	S^* in sample (μ g.)	Urine (g.)	S^* in sample (μ g.)	Urine (g.)	S^* in sample (μ g.)
0-10	0.630	5.8	0.900	7.0	0.270	1.3
10-20	0.491	20.3	0.244	13.4	0.280	7.4
20-30	0.607	22.5	0.251	32.5	0.165	9.0
30-60	4.494	59.9	0.503	54.9	0.357	29.9
60-120	2.923	168	0.241	30.7	0.984	99.4
120-180	4.880	182	c. 0.5	88.7	1.131	129
180-240	2.250	154	0.123	7.6	0.941	81.5
240-300	—	—	0.048†	2.8†	0.292	24.9
300-360	—	—	—	—	0.127	14.4
360-415	—	—	—	—	0.467	31.7
Totals	16.277	612.5	2.8	237.6	5.014	428.5

† These figures relate to the time interval of 240 to 273 min. only.

Rabbits K, L and M received injections of 2800, 2200 and 2500 μ g. S^* respectively.

Table 5. *S^* in the contents of the alimentary canal of rabbit after the intravenous injection of 5 mg. of H^* /kg. of body weight*

	Total S^* (μ g.)	
	Rabbit M (without biliary cannula)	Rabbit J (with biliary cannula)
Stomach	12.4	15
Duodenum	38.5	1.5
Small intestine	57.7	16.5
Large intestine, caecum and rectum (+ faeces passed)	199	—
Bile of rabbit with biliary cannula	—	200

Rabbits M and J received injections of 2500 and 2700 μ g. of S^* , and were killed 7 hr. and 3½ hr. after the injections respectively.

Analysis of the injected pinna of rabbit K showed the presence of less than 1 μ g. of S^* in the whole ear 4 hr. after the injection of 14,000 μ g. of H^* (i.e. 2800 μ g. of S^*). The S^* in the contents of the alimentary tracts of rabbits J and M are given in Table 5.

DISCUSSION

In the experiments described here an effort has been made to trace the H^* injected into rabbits by determining the amount of S^* in the blood, tissues, urine and bile at various intervals after the injection. Because of the limited supply of radioactive S available to us, many of these injection experiments have had to be carried out on single rabbits, but in all cases where an experiment has later been repeated on another rabbit, results have been obtained very similar to those of the earlier experiment. Individual differences do undoubtedly occur, due in some cases perhaps to different rates of excretion of relatively large amounts of S^* in the urine, bile, etc., but there is a very general similarity between the results of similar experiments.

The use of an isotopic tracer in these experiments can, of course, only provide information about the fate of that part of the molecule containing the tracer. The results presented in this paper do not, therefore, give any information about the exact nature of the compound in which the S^* occurs in the tissue; they merely give the amount of free plus combined H^* and its S^* -containing derivatives in the case of the total S^* values, and the amount of S^* (or H^* -‘residues’) firmly attached to the proteins and other ether- and acetone-insoluble constituents of the tissues in the case of the ‘fixed’ S^* values. In view of the fairly rapid hydrolysis of H in aqueous media, it seems likely that most of the ‘free’ S^* (the difference between the total S^* and the ‘fixed’ S^*), particularly that in the tissues several hours after the injection, is largely represented by hydrolysis products, e.g. TG^* (thiodiglycol*) and perhaps H^*-TG^* compounds, rather than by unchanged H^* . The possibility of unchanged H^* remaining associated with the fats and related substances for some considerable time must not be excluded however, although most of our experiments have shown that the tissues of the injected rabbits often lost relatively little of their S^* as a result of long extraction with ethanol, acetone and ether.

Three main conclusions can be reached from the results of the various experiments described in this paper. (a) Much of the injected H^* or its hydrolysis or reaction products rapidly diffuses from the blood stream. (b) There is widespread distribution of S^* in the tissues of the injected animal, with retention of the ‘fixed’ S^* in these tissues for some considerable time after the injection; however, there does not appear to be any obvious relationship between the ‘fixed’ S^* and the extent of damage occurring in any particular tissue. (c) There is rapid excretion, starting shortly after the injections, of S^* -containing compounds (and possibly some unchanged H^*) in the urine and bile.

The appearance of S^* in the urine 10 min. after the injection of H^* indicates a rapid diffusion of H^* from the blood stream, but there is also other evidence of the rapidity of this diffusion. 20 min. after the injection of H^* into rabbit F (Fig. 2) the total S^* content of the blood was only about one-tenth of that which would be obtained by uniform distribution of the H^* throughout the blood, assuming a blood volume of 1/13 of the body weight. In this experiment the blood S^* content remained approximately constant for the period 20 min.–1 hr. after the injection and then fell appreciably during the next 3 hr., largely as a result of a fall in plasma S^* ; for the next 8 hr. the blood total S^* remained fairly constant. Throughout most of the 12 hr. experiment with rabbit F the plasma S^* accounted for about two-thirds of the total blood S^* .

The widespread distribution of S^* in all the examined tissues of the injected rabbits, and the fact that most of this S^* is 'fixed', suggest that there is a general combination of the H^* , or some 'metabolic' product of H^* , with constituents of the tissues. H readily reacts *in vitro* with a variety of proteins (for a short review of the literature, cf. Banks, Bournsell, Francis, Hopwood & Wormall, 1946 *a, b*), and there appears to be no doubt that a similar reaction occurs *in vivo*. The general similarity between the total S^* and the 'fixed' S^* contents of any one tissue at any particular time after the injection of H^* indicates that much of the S^* in the tissue is, in fact, firmly bound to the ethanol- and ether-insoluble constituents of the tissues. A small fraction only of the S^* contents of the organs and tissues examined, particularly in the case of kidney, lung and liver, can be attributed to the blood present in the tissue. The amount of S^* in the kidney, lung and liver 1 hr. after the injection of H^* was, for example, appreciably greater than the S^* content of an equal weight of the blood, and certainly many times as great as the maximum amount of S^* which could be present in the blood in the tissues. In two experiments a kidney of the injected rabbit was perfused for about 10 min. with Ringer solution via the blood vessels, and even after this treatment a high value was obtained for the S^* content of the kidney. There appears to be little doubt therefore that appreciable amounts of S^* are firmly fixed in most tissues after the injection of H^* .

Of the tissues examined, the kidney, lung and liver invariably showed much higher S^* contents than did the others, and it is very significant that these are excretory organs. It would appear that the relatively large amount of S^* in these three tissues might be due to the combination of H^* or H^* -TG* with proteins, etc. during the process of excretion of these substances in the urine and bile and possibly expired air. The rapid excretion of S^* in the urine of the injected rabbits certainly suggests that the

glomerular filtrate might contain significant amounts of unchanged H^* which could of course react with the cells of the tubules. In the case of lung tissue, the high value might be due to the fact that the intravenously injected H^* meets its first major capillary system in the lung; it is much more probable, however, that it is due to the continued passage of H^* , and possibly other relatively volatile S^* -containing compounds, from the blood, and their fixation in alveolar tissue. The high S^* content of the liver can also be explained by reference to the excretory function of the organ, for considerable amounts of S^* were present in the bile of the injected animals. The presence of large amounts of S^* in the excretory organs does not appear to be due solely to a relatively large blood supply, since the thyroid, which has a very large blood supply, shows S^* values much lower than those of kidney, lung and liver.

These experiments have shown that with most tissues there was not much difference between the total S^* and the 'fixed' S^* of that tissue at any one time after the injection. This 'fixed' S^* does not appear to be permanently fixed, and at a later stage (cf. Tables 1 and 3 and Fig. 1) there was appreciable loss of this S^* . Values for the 'fixed' S^* contents of the tissues 12 hr. after the injection are not available, but it will be noted that the total amount of S^* /g. of dried kidney, liver or lung tissue 12 hr. after the injection was lower, and generally much lower, than the lowest 'fixed' S^* content of the same tissue during the period 1–4 hr. after the injection. For example, the total S^* content of the kidney 12 hr. after the injection of H^* was 3.4 $\mu\text{g./g.}$ of dried tissue, whereas the 'fixed' S^* contents 1–4 hr. after the injection ranged from 10.5 to 21 $\mu\text{g./g.}$ How this 'fixed' S^* is removed from the living tissue is not known, but it is interesting to note that *in vitro* experiments with H^* and plasma have suggested the possibility of some loss of S^* by H^* -plasma-protein complexes when these are kept in solution at pH 7.5 and 37° (Banks *et al.* 1946 *a*). On the other hand, the loss of 'fixed' S^* by the tissues may simply be due to the removal of the changed protein, i.e. the H^* -protein, from the tissue.

The comparison of the total S^* contents of any one organ 1, 2½, 4 and 12 hr. after the injection of H^* into rabbits has shown that with kidney and liver the maximum values are recorded 1 hr. after the injection, and that there is a subsequent pronounced fall (cf. Fig. 1). In the case of lung, however, the value rises to reach a peak only at the 4 hr. stage, with a distinct fall during the next 8 hr. (i.e. the value for the lung appears to be rising while those for the kidney and liver are falling); if the high S^* contents of kidney and lung are both to be explained on the 'excretory hypothesis' it is not easy to explain the differences between the times required for the maximum values to be attained.

The total S* in the bone marrow was very small at all times, i.e. at 1, 2½, 4 and 12 hr. after the injection, and we have been unable to confirm the observation of Salter (1942) that the S* content of the bone marrow 3 hr. after the injection of H* is greater than the S* content of the kidney, liver and lung. In our experiments the bone marrow showed a maximum S* content at 1 hr., but the values were very low compared with those for kidney, liver and lung. The S*/total-N ratio for bone marrow was, however, of the same order as those for most other tissues of the body, and this would support the view that most of the S* in the various tissues is in association with proteins. There does not appear to be any preferential storage of injected H*, within the limits of our experiments, in the fatty tissues, and in several cases the removal of fatty matter from the S*-containing tissue has led to an increase in the S* content per g. of dried tissue.

The other tissues examined (Table 2) were found to have S* contents appreciably below those for the excretory organs; the total S* values ranged from 0.2 to 0.7 µg./g. of wet tissue (or 0.9 to 3.1 µg./g. of dried tissue). Duodenal wall gave the highest value among these other tissues (4.7 µg./g. of dried tissue), but this high value can probably be explained by the excretion of much S* in the bile and its retention in the duodenal wall or mucosa.

The excretion of S* in the urine following the injection of H* into rabbits starts very soon after the injection, and with rabbits K, L and M the excretion of S* in the urine continued during the whole of the time that the animals were kept alive (Table 4, Fig. 4). The possibility that some of this S* excreted during the first 10 or 20 min. after the injection might have been present as H* cannot be excluded, and the passage of a reactive substance of this type along the renal tubules would undoubtedly account for the relatively high S* content of the kidneys of the injected rabbits. The amount of S* excreted in the urine during the course of these experiments varied from one rabbit to another, the amount being largely dependent on the rate of excretion of urine. With rabbits L and M the rate of urine flow fell in the later stages of the experiment and the urinary excretion of S* fell correspondingly. In these experiments every effort was made to promote secretion of urine by the prior administration of water and by the injection of saline during the experiment, but the total volume of urine secreted by two of the rabbits was rather small. The total amounts of S* excreted in the urine by rabbits K, L and M were respectively 21.9, 10.8 and 17.1 % of that injected, and there is good reason for assuming that these values would have been increased very considerably had the animals been kept alive longer; the last samples of urine obtained from all three rabbits showed no appreciable falling

off in S* content. It certainly is interesting to note that two of the rabbits excreted in the urine about one-fifth of the total amount of the S* injected as H*.

The non-anaesthetized rabbit (A) excreted 121 µg. of S* in the 3 ml. of urine found in its bladder 1 hr. after the injection of H*, and this value agrees satisfactorily with the corresponding values for the urine passed during the first hour by rabbits K and L (109 and 108 µg. respectively); only 48 µg. of S* were excreted by rabbit M during this period, but this low excretion was probably due to the low urine excretion (about 1 ml.). This observation with rabbit A indicates that the urinary excretion of S* by rabbits K, L and M cannot be attributed to the effects of the anaesthetic or of the surgical treatment.

Considerable amounts of S* were excreted in the liver bile of an injected rabbit (Fig. 3), and there is much evidence that this may be one of the important channels of excretion for H* and related compounds, just as it is for certain other fat-soluble materials and some drugs, toxins and metals. The H* may be excreted as unchanged H* or its hydrolysis products, and these may be free or associated with the bile salts or bile proteins; the compounds with the bile salts, in the form of complex 'choleic acids' similar to those described by Wieland & Sorge (1916) (cf. also Francis, Hurtley & Town, 1938) might be dissociated in the alimentary tract with the liberation of H* or other reactive S*-containing compounds. In the experiment described here, about 7 % of the S* injected as H* was found in the bile during the 3½ hr. experiment, and about one-sixth of this biliary S* appeared in the first 20 min. and over half during the first hour. This rapid excretion suggests that some of the S* might have been present as unchanged H*, and if this bile had been allowed to pass into the duodenum the H* might have caused appreciable damage to the wall of the alimentary tract; H excreted in the bile might, therefore, be a contributory factor to the intestinal damage occurring in H poisoning referred to by several investigators (cf. Cameron, 1941, 1942). If, on the other hand, the sphincter of Oddi were closed, this H would be diverted into the gall bladder where hydrolysis or possibly reabsorption would occur.

The bile duct is, however, not the only route by which S* enters the alimentary tract of the rabbit following the injection of H*, for about 35 µg. of S* were found in the contents of the stomach and small intestine even when no bile was allowed to enter the alimentary tract; the corresponding figure for the large intestine is unfortunately not available. In this experiment, the bile collected from a cannula inserted into the bile duct contained 200 µg. of S* (in 3½ hr.). In a comparable experiment where the flow of bile into the duodenum was not impeded, the contents of the alimentary tract contained, 7 hr. after the injection, about 300 µg. of S* with about

two-thirds of the total in the faeces and the contents of the large intestine. These results would appear to suggest that a considerable amount of the S* excreted in the bile by the injected rabbits is carried through the alimentary tract without resorption, or that a very large amount is excreted by the large intestine.

It has not been possible in any one of these experiments to attempt to trace the whole of the S* injected as H*, and as many of the experiments were carried out under different conditions, an attempt to prepare a 'balance-sheet' by adding up the results of the different experiments might meet with many objections. However, it is interesting to note that in the first hour after injection, rabbit J excreted about 4% of the injected S* in the bile, and three other rabbits excreted 2-5% in the urine. The amount of S* in the majority of the tissues (muscle, heart, etc.) at the 1 hr. stage was 0.3-0.8 $\mu\text{g./g.}$ of wet tissue and the amount in the blood was usually in the upper regions of this range. Uniform distribution of the injected S* (1000 $\mu\text{g./kg.}$ of body wt.) would require a tissue and blood S* content of 1.0 $\mu\text{g./g.}$ of wet tissue, and the observed values of 0.3-0.8 $\mu\text{g./g.}$ appear to be quite satisfactory if allowance is made (a) for the excretion in the urine, bile, alimentary tract and possibly expired air, (b) for the relatively high S* content of the kidneys, lung and liver (1.6-4.0 $\mu\text{g./g.}$ of wet tissue), and (c) for the weight of the skeleton.

SUMMARY

1. A study has been made, with the aid of the radioactive S³⁵ as a tracer element, of the fate of mustard gas (H) injected intravenously into rabbits.

2. H* (mustard gas containing S³⁵) was injected in amounts of 5 mg. of H*/kg. of body wt. and determinations were made of the S* content of many of the tissues, the urine and the bile at intervals after the injection.

3. The injected material rapidly diffused from the blood stream and appreciable amounts of S*-containing compounds appeared in the urine and bile less than 20 min. after the injection.

4. There was widespread distribution of S* in the tissues of the injected rabbits. Kidneys, lung and

liver gave the highest values of all the tissues examined, and it is suggested that the high values for these three organs are due to their excretory functions.

5. The greater part, and sometimes all, of the S* in the tissues of the injected rabbits was present as 'fixed' S* (not extracted by ethanol, acetone and ether). It is believed that at least part of this 'fixed' S* is present in protein complexes.

6. A comparison of the total S* contents of the tissues 1, 2½, 4 and 12 hr. after the injections showed that with the kidney and liver the highest value was at the 1 hr. stage, whereas with the lung the maximum was not reached until the 4 hr. stage. The results suggest that 'fixed' S* as well as 'free' S* was removed from the tissues during the later part of this experiment.

7. The amount of S* in the bone marrow of these injected rabbits was small, but the S*/N ratio for this tissue was of the same order of magnitude as the corresponding ratios for other tissues.

8. As much as 22% of the S* injected as H* was excreted in the urine by one rabbit and 17% by another, in periods of 4 and 7 hr. respectively after the injection.

9. In another experiment, 7% of the injected S* was recovered from the liver bile during the first 3 hr. after the injection. This mode of excretion might in part account for intestinal damage occurring in H poisoning.

10. No evidence has been obtained of preferential retention of the injected H* or its S*-containing derivatives in the fatty tissues of the body, or in any organ, with the exception of kidney, lung and liver.

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