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

6. THE FATE OF INJECTED $\beta\beta'$-DICHLORODIETHYL SULPHONE AND $\beta\beta'$-DICHLORODIETHYL SULPHOXIDE (CONTAINING RADIOACTIVE SULPHUR) IN THE ANIMAL BODY†

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It has been shown (Boursnell, Cohen, Dixon, Francis, Greville, Needham & Wormall, 1946) that the intravenous injection of *H** (mustard gas containing the radioactive S^{35}) into rabbits is followed by the distribution of S* in most of the tissues of the animal and the retention of part of this S* as firmly combined or 'fixed' S* for some considerable time after the injection. During discussion of this work, Dr M. Dixon suggested that similar experiments with *HO** ($\beta\beta'$-dichlorodiethyl sulphoxide*) might yield interesting results in view of the fact that this substance, the Cl atoms of which are non-reactive, is non-vesicant and does not affect enzymes, and yet is reported to be toxic on injection; there seemed a possibility, therefore, that injected *HO** might give systemic effects without fixation.

In these experiments amounts of *HO** and HO_2* approximately the same as the amount of *H** used in the earlier experiments (Boursnell *et al.* 1946) were injected into rabbits, and the S* contents (total and in most cases 'fixed' S* also) of the blood and some of the tissues were determined 1 hr. after the injections. This has enabled us to make a comparison of the distribution of S* in the tissues following injections of the three substances, *H**, *HO** and HO_2* (an asterisk indicates the presence of the radioactive S^{35} in the substance).

METHODS

Injections. The *HO** and HO_2*, prepared as described previously (Boursnell, Francis & Wormall, 1946) were dissolved in triacetin to give 5·0 and 2·6% solutions respectively, and these solutions were injected into the ear veins of rabbits. Rabbit P (2·4 kg.) received 5·5 mg. of *HO**/kg., and rabbit Q (2·37 kg.) received 5·0 mg. of HO_2*/kg. of body wt., and both animals were killed 1 hr. after the injections.

Collection and treatment of the blood and tissue samples, and S* *determinations.* These were carried out as described elsewhere (Boursnell *et al.* 1946). In addition, for 'fixed' S* determinations on blood plasma and cell samples, the solution or suspension was poured into 6 vol. of ethanol (usually after the cells had been laked with a little water), and centrifuged; the precipitates were washed twice with ethanol and once with acetone (about 5–7 ml. of solvent each time) and finally Soxhlet-extracted (for four 3 hr. periods, with ether and acetone in rotation).

An S* determination was made on the gall bladder (plus its contents) of rabbit P, but no S* determination could be made on the bile of rabbit Q as the gall bladder of this animal was found to be empty. The latter rabbit had, however, about 5 ml. of urine in its bladder at death and an S* determination was made on this urine.

For the 'fixed' S* determinations the tissues were exhaustively extracted with acetone and ether as described previously (Boursnell *et al.* 1946).

RESULTS

The results of the experiments with *HO** and HO_2* are given in Tables 1 and 2 respectively. The total S* contents are recorded as μg. of S*/g. of wet tissue and also per g. of dried tissue, and the N contents of the acetone and ether extracted tissues and some of those for the dried unextracted tissues are given, in order that an estimate might be obtained of the maximum protein content of the preparations. For comparison purposes, some of these results and some of the corresponding values obtained in the experiments with *H**-injected rabbits (Boursnell *et al.* 1946) are grouped together in Table 3.

The rabbit injected with *HO** was found to have 19 μg. of S* in its gall bladder plus contents, and the rabbit injected with HO_2* had 448 μg. of S* in the 5 ml. of urine in its bladder 1 hr. after the injection. The part of the ear into which the intravenous injection of HO_2* (11·8 mg. of HO_2*, containing 1980 μg. of S*) was made, was found to contain only 3 μg. of S* when the rabbit was killed.

DISCUSSION

Although these observations have had to be made, because of limited supplies of S*, on single rabbits (two in the case of *H**), it is believed that the values obtained are sufficiently typical to warrant a general comparison of the fates in the animal body of intravenously injected *H**, *HO** and HO_2*. The following general conclusions can be reached from the results summarized in Table 3.

† This investigation was the subject of reports to the Ministry of Supply in 1944–5.

Table 1. S* *contents of rabbit tissues* 1 *hr. after the intravenous injection of* 5·5 *mg. of HO*/kg. of body weight*

	Total S* (μg./g.)		Extracted tissue	
	Dry tissue	Wet tissue	N (%)	'Fixed' S* (μg./g. dry tissue)
Kidney	18·5	4·4	12·9	22·3
	23·4	5·5	12·7	20·7
Liver	7·1	2·3	13·5	7·3
	—	—	13·2	7·9
Lung	4·7	1·5	13·7	10·2
	7·0	1·7	13·3	8·2
Muscle (skeletal)	0·8	0·2	—	0·2
	0·9	0·3	13·7	0·0
Stomach wall†	1·5	0·3	13·2	1·6
	1·4	0·4	13·1	1·8
Brain	2·6	0·6	—	—
Duodenal wall†	11·2	2·2	—	—
	7·6	1·7	—	—
Spleen	0·3	0·1	—	—
Bone marrow	1·0	0·7	—	—
Blood plasma‡	—	0·6	—	—
	—	0·6	—	—
Blood cells‡	—	0·1	—	—
	—	0·04	—	—

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

‡ The values for blood fractions are in terms of μg. of S*/ml. of whole blood.

Table 2. S* *contents of rabbit tissues* 1 *hr. after the intravenous injection of* 5 *mg. of* HO_2**/kg. of body weight*

	Unextracted tissue			Extracted and dried tissue	
	N in dry tissue (%)	Total S* Dry tissue (μg./g.)	Total S* Wet tissue (μg./g.)	N (%)	'Fixed' S* (μg./g.)
Kidney	10·5	23·2	5·8	12·6	46
	11·5	37·6	9·2		38
Liver	8·4	1·2	0·37	9·5	1·1
	8·4	0·94	0·3		1·4
Lung	9·9	7·9	1·9	12·8	12·1
					15·7
Muscle (skeletal)	10·6	0·8	0·4	14·8	1·9
					1·6
Stomach wall	10·4	2·5	0·59	13·1	4·4
					5·2
Brain	7·5	4·4	0·94	9·0	8·4
Duodenal wall	9·1	1·6	0·25	12·8	3·8
Bone marrow	2·0	0·2	0·15	—	—
Heart	10·6	3·4	0·75	13·4	5·6
Blood plasma†	—	—	0·36	—	0·14
			0·31		0·16
Blood cells†	—	—	0·13	—	0·10
					0·11
Blood cells and plasma†	—	—	0·47	—	0·26

† The values for blood fractions are in terms of μg. of S*/ml. of whole blood.

Table 3. *Comparison of* S* *contents of rabbit tissues* 1 *hr. after the intravenous injection of H*, HO* and* HO_2***

	H†*	*HO**	HO_2*
	Total S* (μg./g. wet tissue)		
Kidney	4	5·0	7·5
Liver	1·6	2·3	0·34
Lung	1·6	1·6	1·9
Muscle	0·5	0·25	0·4
Stomach wall	0·5	0·35	0·59
Duodenal wall	0·86	2·0	0·25
Heart	0·7	—	0·75
Brain	0·3	0·6	0·94
Bone marrow	0·5	0·7	0·15
	'Fixed' S* (μg./g. dry tissue)		
Kidney	18·5	21·5	42·0
Liver	6·2	7·6	1·2
Lung	5·2	9·2	13·9
Muscle	0·9	0·1	1·8
Stomach wall	0·6	1·7	4·8
Duodenal wall	6·0	—	3·8
Brain	3·0	—	8·4
	Total S* (in terms of μg./ml. of whole blood)		
Blood cells	0·4	0·07	0·13
Blood plasma	1·0	0·6	0·33
Blood (cells + plasma)	1·4	0·67	0·46
	'Fixed' S* (in terms of μg./ml. of whole blood)		
Blood cells	—	—	0·1
Blood plasma	—	—	0·15
Blood (cells + plasma)	—	—	0·25

To simplify comparison, average values are given, in the above table, for the *H**-injected rabbits, and in all cases where duplicate determinations were made on any organ or tissue.

† Values from Boursnell *et al.* (1946).

(*a*) The injected compounds (*H**, *HO** and HO_2***) did not remain long in the blood stream; they were rapidly distributed throughout the body, and 1 hr. after the injection significant amounts of S* were found in all the tissues examined.

(*b*) The amount of S* in the blood 1 hr. after the injection was of the same order for all three substances. The S* remaining in the blood at this stage was approximately one-thirtieth of that injected.

(*c*) The total S* contents of the majority of the tissues of the injected rabbits were of the same order (usually between 0·3 and 0·9 μg./g. of wet tissue), with no general difference between those following the three different injections. The values for the excretory organs, kidney, lung and liver, were on a considerably higher level, except in the case of the liver of the HO_2***-injected rabbit. The total S* contents for the kidney were in all cases easily the highest for any of the tissues examined, being five to twenty times those for such tissues as muscle, heart, brain or bone marrow.

(*d*) The firmly combined or 'fixed' S* contents of the tissues were approximately the same with *HO**-injection as with *H**-injection, but the values for the HO_2-injected animals were distinctly higher (except again for the liver) than the values for the corresponding tissues of the *H**- or *HO**-injected rabbits.

(*e*) There was fairly rapid excretion of S* in the urine and/or bile after the injection of all three substances, possibly in both urine and bile in all cases; no information is available, however, about the biliary S* for the HO_2*-injected rabbit or the urinary S* for the *HO** rabbit. This excretion of S*-containing compounds (possibly including some *H**, *HO** or HO_2*) almost certainly accounts for the occasional high S* contents of the duodenal and stomach walls of the injected rabbits.

It is rather surprising that *H* and its oxidation products, *HO* and HO_2, should, when injected into the animal body, all behave similarly, at least as far as distribution in and combination with the tissues and excretion from the body are concerned. These three compounds have very different solubilities in water, and chemically they show very different reactivities. HO_2 and *H* readily react with a variety of proteins (for a short review of the literature, cf. Banks, Boursnell, Francis, Hopwood & Wormall, 1946*a*, *b*), the former by virtue of a reaction with the amino-groups and to a lesser extent with SH groups of the protein, and the latter by reaction with COOH (Northrop, 1942; Bergmann, 1942), with iminazole (Moritz, 1942; du Vigneaud, 1942) and possibly other groups such as SH (Peters & Wakelin, 1941). *HO**, on the other hand, does not react with proteins under physiological conditions of pH and temperature (Bailey & Webb, 1944; Banks *et al.* 1946*b*).

The presence of appreciable amounts of S* in all the tissues of the injected animal 1 hr. after the injection of *H** and HO_2* could readily be explained by suggesting that the injected material has reacted with the tissue proteins, for both reagents readily react *in vitro* within this period of time with plasma and other proteins to produce complexes from which the S* cannot be removed by prolonged extraction with acetone and ether. In the *in vitro* experiments with plasma and small equivalent quantities of *H** and HO_2* (with an *H** or HO_2* to protein ratio of the same order as, but perhaps a little less than, that which would occur in the blood stream of the injected animals immediately after the injections) the reaction with the proteins was complete in about 30 and 60 min. respectively, and the S* content of the final HO_2*-proteins was about $1\frac{1}{2}$ times that of the final *H**-proteins (Banks *et al.* 1946*b*). It seems therefore quite feasible that the presence of 'fixed' S* in the tissues after the injection of *H** and HO_2* is due to the combination of the injected material with the body proteins; the lower 'fixed' tissue S* contents after *H** injection compared with those after HO_2* injection are most probably largely due, as is the similar difference between the S* contents of the *H** and HO_2* plasma proteins in the *in vitro* experiment, to the 'loss' by hydrolysis of an appreciable fraction of the *H** before it has time to react with the proteins.

*HO**, however, does not react with proteins *in vitro*, and the fixation of S* in the tissues of the *HO**-injected animals cannot presumably be attributed to a direct reaction with the general tissue proteins. A reaction with some specially reactive tissue protein might be postulated, but there is no evidence of this. In view of the general similarity between the amounts of 'fixed' S* in the tissues following the injection of the closely related compounds *H**, *HO** and HO_2* one is tempted to believe that the same type of reaction might be concerned in all three cases. If this is true, it would exclude the possibility of a reaction with proteins in every case, unless it can be shown that *HO** is converted in the body into some more reactive substance (e.g. by oxidation to HO_2* or reduction to *H**). Further experiments will be necessary to determine whether or not *HO** is metabolized in this way, and to test the validity of several other hypotheses which can be offered. One possibility is that *H** and *HO** are both oxidized in the body to HO_2*, but the conversion of *H* into HO_2 requires an oxidation potential considerably higher than that which is encountered in animal tissues (Sugden, 1941), and in any case, it seems fairly certain that an appreciable part of the injected *H** must, by analogy with the *in vitro* experiments, combine fairly quickly with the plasma and tissue proteins. The fact that there are certain differences between the systemic effects produced by injected *H* and those produced by injected *HO*, e.g. the inhibition of glycolysis in bone marrow by *H* but not by *HO* (Dixon, 1944) suggests that these two substances may differ very considerably in their general *in vivo* action on the tissues. It may be, therefore, that the fixation of S* in the tissues after the injection of *HO** is effected by a process quite different from that operating in the case of *H** and HO_2*.

It is interesting to note that the total S* content of the blood 1 hr. after the injection of *HO** was of the same order as that 1 hr. after the injection of *H** or HO_2*, with over two-thirds of the blood S* in the plasma in each case. It seems probable that some of this S* was present, in the case of the injections with *H** and HO_2*, as *H**-proteins and HO_2*-proteins respectively, but there was also, in the latter case at least, an appreciable amount of 'free' S* (total S* minus 'fixed' S*) in the blood; the corresponding values for the *H**- and *HO**-injected rabbits are not available. This 'free' S* probably represents a mixture of various acetone- or ether-soluble S*-containing compounds and a further study of these S*

compounds in the blood after the injection of H^*, HO_2^* and HO^* might help to throw light on some of the problems discussed in this paper.

SUMMARY

1. The fate in rabbits of intravenously injected HO ($\beta\beta'$-dichlorodiethyl sulphoxide) and HO_2 ($\beta\beta'$-dichlorodiethyl sulphone), two oxidation products of mustard gas (H), has been studied with the aid of the radioactive S^{35} as a tracer element. (An asterisk indicates the presence of S^{35} in the substance.)

2. The injected HO^* or HO_2^* was fairly rapidly distributed throughout the body, and appreciable amounts of S* were found in all the tissues examined 1 hr. after the injections. As in the corresponding experiments with H^*, the kidney and lung showed much higher S* contents than did the other organs and tissues.

3. Appreciable quantities of S* were excreted in the urine and/or bile after the injection of HO^* or HO_2^*, as in the case of H^*.

4. A comparison of the results of the experiments with H^*, HO^* and HO_2^* suggests that the three substances are similarly distributed throughout the body after injection, and that they all react with the tissues to form acetone- and ether-insoluble S*-containing compounds ('fixed' S*). This reaction may be partly or wholly concerned with the tissue proteins in the case of H^* and HO_2^*, but probably not with HO^*, for this compound, unlike H^* and HO_2^*, does not react or combine with proteins in *in vitro* experiments carried out under physiological conditions. The possibility of an *in vivo* conversion of HO^* into some more reactive compound is tentatively discussed.

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

7. THE IMMUNOLOGICAL PROPERTIES OF PROTEINS TREATED WITH MUSTARD GAS AND SOME RELATED COMPOUNDS†

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It has been shown (Berenblum & Wormall, 1939) that mustard gas (H) and its sulphone, $\beta\beta'$-dichlorodiethyl sulphone (HO_2), react with serum proteins at room temperature and at about pH 8 to give protein derivatives with a new immunological specificity. Injection of the H-treated horse serum proteins into rabbits produced antibodies which reacted with H-treated rabbit (or chicken) serum proteins, and the antibodies to HO_2-proteins showed a specificity characteristic for HO_2-proteins. These results showed that H and HO_2 had effected significant chemical changes in the protein molecule, and the absence of serological cross-reactions suggested that the two reactions might be dissimilar. Evidence was obtained that the chemical changes effected were

† This investigation formed the basis of reports to the Medical Research Council and to the Ministry of Supply in 1940.