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

1. GENERAL INTRODUCTION AND ACKNOWLEDGEMENTS

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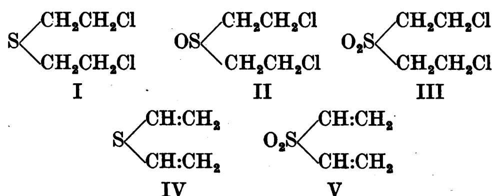
The investigations described in this and the following seven papers were started early in 1939, partly as an extension of the study of the immunological properties of mustard gas-treated proteins (Berenblum & Wormall, 1939) and partly as a more general study of the action of mustard gas (H) on proteins and other tissue constituents, with particular reference to its vesicant action. The possibility that H is oxidized in the tissues or that it undergoes some other change (e.g. loss of HCl to form unsaturated compounds) was kept in mind, and in many cases experiments parallel to those made with H (I) were carried out with the following closely related compounds: HO ($\beta\beta'$-dichlorodiethyl sulphoxide, II); HO_2 ($\beta\beta'$-dichlorodiethyl sulphone, III); divinyl sulphide (IV); divinyl sulphone (V).



The evidence available prior to 1939 appeared to suggest that H is not a very reactive substance under physiological conditions of pH and temperature, though it was known that it had a destructive or inhibitory action on tissue glycolysis (Berenblum, Kendal & Orr, 1936). Several theories or suggestions had been put forward to account for the vesicant action of H, but the experimental support for these theories was not very strong. An early suggestion was that the chief factor might be HCl liberated inside the cell by the hydrolysis of H which had penetrated through the cell membrane. There appears, however, to be no close correlation between acid liberation and the vesicant action of H and its sulphone (Peters & Walker, 1923), and also, it is rather difficult to believe that the buffering power of the cells and the tissue fluids would be incapable of dealing with the acid liberated from the small amounts of H which effect vesication. Furthermore, many other substances which liberate HCl in a similar way are non-vesicant. The possibility that acid liberation may be a contributory factor to the disturbance in the equilibrium in the cell cannot, however, be excluded.

Another theory which had attracted much attention arose from the suggestion of Flury & Wieland (1921), that H is converted into the corresponding sulphoxide (HO) and sulphone (HO_2) by tissue oxidases, and that these oxidation products are largely responsible for the toxic action of H on the cell. Although there was little direct experimental evidence to support it, this theory (usually referred to as the 'sulphone theory') had many attractive features. Peters & Walker (1925) found that HO_2 was as strongly vesicant as is H when it is properly applied, and workers at H.M. Experimental Station, Porton, observed that it was very toxic on injection (cf. also Kuchárik & Telbisz, 1939, who reported greatly enhanced vesicant activity by HO_2 when the substance is injected subcutaneously). In connexion with this 'sulphone theory', Ford-Moore (1940) suggested that HO_2 is converted into divinyl sulphone before it reacts *in vitro* with amino-groups, and Ford-Moore & Lidstone (1940) gave support to the theory by pointing out that no sulphide is vesicant unless it is capable of yielding, on oxidation, a sulphone which can lose one or two molecules of HCl (or HBr) to give a compound of the type $R.SO_2.CH{:}CH_2$.

The immunological evidence of Berenblum & Wormall (1939) showed, however, that H has a direct action on serum proteins at ordinary temperatures and at about pH 8, and that the product obtained differs very significantly from HO_2-treated serum proteins. There was also a certain amount of other evidence available at that time to show that H can react directly with tissue constituents under physiological conditions, and later work, by ourselves and other authors, on the action of H on proteins, and more particularly the evidence of Peters and his colleagues at Oxford and Dixon and his colleagues at Cambridge, that H itself inhibits certain tissue enzymes, showed that it was not necessary to postulate the transformation of H into

† This work was initiated as a joint investigation by the two departments concerned and was later continued (1941–5) as part of a programme of Extra-Mural research carried out for the Ministry of Supply by a team under the leadership of Prof. Wormall.

HO_2 or some other oxidation product to account for its toxic action. Most attention has therefore been directed, in our investigations, to the reactions of H, but for comparison purposes, parallel experiments have usually been made with HO_2 and sometimes with HO, particularly in our earlier experiments, which were carried out at a time when there was still considerable support for the 'sulphone theory'. Although it is not our intention to discuss the evidence for and against this theory, it should be pointed out that Sugden (1941) reached the conclusion that the conversion of H into HO_2 in the tissues is very improbable, since this change requires an oxidation potential considerably higher than that likely to be reached by any tissue oxidase system.

Early in 1939 we decided to make use of isotopic tracers for our investigations, and we considered the possibility of studying the *in vitro* and *in vivo* action of H and related compounds on proteins and animal tissues with the aid of H containing one or more of the following isotopes: radioactive S (S^{35}), radioactive Cl (Cl^{36} and Cl^{38}) and deuterium. Cl^{36} and Cl^{38} are not very satisfactory for biological work of the type envisaged due to their rates of decay. The latter has a relatively short half-life period of 37 min., thus limiting the time during which the H will retain a useful radioactivity, and the very long half-life period (71 yr.) of Cl^{36} makes this isotope difficult to prepare in suitable quantities. Moreover, mustard gas (H) readily loses HCl by hydrolysis, and this HCl, and any liberated from a reaction between H and amino-groups, etc., might be rapidly excreted from the animal body, or might combine with substances other than those which had reacted with H. Thus it was thought that the determination of the fate of the Cl of mustard gas would provide little information about the action of this compound on animal tissues.

It was decided, therefore, that S^{35} (of half-life period 88 days) and deuterium would probably be the most suitable isotopes for this work, and the preparation of H containing one or both of these isotopes was undertaken. It was hoped that work with 'deutero-radio'-H would be possible, for there are distinct advantages in the use of a substance containing two different isotopic tracers, but, for several reasons, we were unable to carry out our original plan; 'deutero'-H (H containing about 99% of its hydrogen replaced by deuterium) has been prepared, but so far we have not used it for our investigations.

Information about S^{35} was somewhat scanty in 1939. Anderson (1936) and Tuck (1939) described the production and separation of this radioactive isotope by the irradiation of carbon tetrachloride with neutrons from a radon-beryllium source, and Voge & Libby (1937) had obtained the isotope by the same neutron reaction (using a cyclotron source) and also by the bombardment of roll-sulphur with deuterons. The β-rays emitted by S^{35} are, however, of low penetrating power, and this was a big obstacle to quantitative work with the isotope; Voge (1939), for example, describing exchange reactions with S^{35}, stated that 'no attempt was made in the experiments reported to determine activities precisely, but it is thought that quantitative experiments could be made with strongly active samples, using "infinitely thick" layers' (cf. also Voge & Libby, 1937).

In our earlier investigations we were limited to small supplies of S^{35} obtained with the aid of radium-Be as a source of neutrons, but in 1940 and 1941 we were able to irradiate carbon tetrachloride with fast neutrons from the Cambridge cyclotron. This latter method, and the use of highly sensitive Geiger-Müller counters (cf. Banks, 1946), enabled us to prepare H^* (mustard gas containing S^{35}) with a sufficiently high degree of radioactivity for our biological investigations. The details of the preparation and separation of radioactive sulphur (S^*) and the method of preparation of H^* and related compounds, and the determination of S^* in biological material are described elsewhere (Banks, 1946; Boursnell, Francis & Wormall, 1946); the results of our experiments on the preparation of the isotope were essentially similar to those recorded independently by Kamen (1941). At a later stage of the investigations (1943–5) we were able to obtain from the U.S.A., through the Ministry of Supply, supplies of S^* of much greater radioactivity than those which had hitherto been available to us, and we were thus able to undertake, in collaboration with Dr Dixon's team, the injection experiments reported in papers 5 and 6 of this series.

At the same time that these *in vitro* and *in vivo* investigations with H^* were being carried out, a further extension of the immunological work was made. For this purpose a more complete study of the action of H and HO_2 on amino-acids and proteins was undertaken, in the hope that the results might throw some light on the action of H on tissue proteins, and possibly on the phenomenon of hypersensitivity, a condition which occasionally arises in individuals who are exposed to H. Although it was unlikely that a solution to this problem of hypersensitivity might be found in desensitization, it was thought that a more detailed knowledge of the immunological reactions occurring in the tissues of the animal exposed to H might yield useful information about some of the chemical processes associated with the vesicant and toxic action of H, HO_2 and related compounds. For a similar reason, a study was made of the action of H on complement, a very labile system, possibly partly enzymic in nature, present in the blood plasma. It was found that small quantities of H readily destroy the specific haemolytic activity of this system, and a further extension of this work to include a general study of

the action of many widely differing vesicants on complement was contemplated, but has not yet been carried out.

The results of our investigations with 'radio-H' (H^*) have fully confirmed the view that H reacts, under physiological conditions of pH and temperature, with a variety of proteins forming protein complexes whose chemical and physical properties are considerably different from those of the original protein. The rate of combination of H with serum and other proteins is rapid, and the total amount of H which can combine with the proteins is quite adequate to account for the marked change in immunological properties effected by the treatment of serum proteins with H.

The injection experiments with H^*, HO^* and HO_2^* have shown that appreciable amounts of the injected compounds enter into combination with widely differing tissues of the animal body. There is a general similarity between the distribution of S^* in the tissues after the injection of all three compounds, and the 'fixation' of S^* in the tissues after the injection of H^* and HO_2^* might conceivably be due to the combination of the injected compound with the tissue proteins; this explanation would not account, however, for the results with injected HO^* unless it is assumed that HO^* is converted, in the tissues, into HO_2^* or some other compound which is capable of combining with proteins. The injection of H^*, HO^* and HO_2^* is followed, in each case, by the rapid excretion of considerable amounts of S^*-containing compounds in the urine and/or bile.

The immunological and chemical investigations with ordinary H and radio-H and related compounds have shown that there is a very marked difference between the action of H and that of HO_2 on proteins. Although the results do not exclude the possibility that some H may be converted into HO_2 in the animal body, they suggest that the marked biological effects of H might readily be explained by a direct action of H on the proteins and related constituents of the tissues. Support has been obtained, however, for the view that HO_2 is converted into divinyl sulphone before it reacts with amino-acids and proteins.

During most of the investigations described in the following papers we have had frequent discussions with Dr M. Dixon and members of his research team in Cambridge, and with Prof. R. A. Peters, and we are glad of this opportunity to express our sincere thanks to them all for the help we have derived from these discussions. Some joint investigations were made with members of Dr Dixon's team, and these are described in one of the following papers or are referred to in publications by Dr Dixon and his colleagues.

Our earlier work with radioactive sulphur would not have been possible without the help of Prof. N. Feather and Dr E. Bretscher and their colleagues in the Cambridge cyclotron and H.T. laboratories. We are also grateful to Prof. Sir James Chadwick for making arrangements whereby a supply of S^{35} was obtained from Liverpool in September 1942. Our later supplies of S^{35} came from the U.S.A., and we wish to thank Prof. E. O. Lawrence and all those at the Radiation Laboratory, Berkeley, California, who were concerned with the preparation of this material. Our thanks are also tendered to Prof. F. C. Henriques for a sample of highly radioactive H^* which he sent to Dr Dixon. We should like to take this opportunity of expressing our gratitude to Mr L. T. D. Williams, of the Ministry of Supply, for having made the necessary negotiations in the U.S.A., and we also wish to thank Mr J. Davidson Pratt and his colleagues at the Ministry of Supply for their help and support. We are indebted to the Director-General of Scientific Research (Defence), Ministry of Supply, for permission to publish the results of investigations carried out by us for the Ministry.

Most of the investigations described in the following seven papers were carried out after the evacuation of the Preclinical part of our Medical College to Cambridge on the outbreak of war. Research accommodation was provided for us in the Sir William Dunn Institute of Biochemistry, and later in the Molteno Institute of Parasitology. It is with sincere gratitude that we thank Prof. Sir Frederick Gowland Hopkins, Prof. A. C. Chibnall and Prof. D. Keilin for this accommodation and for the very generous help which they and their colleagues have at all times given to us.

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