ferred can scarcely affect our conclusion: that if mutagens induce different ratios of visibles to lethals in the same sample of treated chromosomes, their mode of mutagenic action is somehow different.

(4) *Mode of mutagenic action of 2-chloroethyl methanesulphonate.* Our interpretation[8] of the mode of mutagenic action of 2-chloroethyl methanesulphonate is based on the comparative study of the chemical reactivity and mutagenicity of the above compound and some closely related alkyl-methanesulphonates. The mutagenic criteria considered were gross trends and points of detail were only mentioned as complementary evidence. Our argument rests on the complete reversal of the mutagenic cell-stage response under the effect of 2-chloroethyl methanesulphonate as compared with that of other alkyl methanesulphonates including the ethyl ester (see Fig. 2, ref. 8). As clearly stated in our contribution[8], and has been shown experimentally[9], changes in the brood-mutation pattern do occur with some compounds, due to cells being killed by the treatment, variation in the speed of differentiation of the germline, or on altering the experimental procedure, particularly the brood period. These changes, however, are of a much lower order than the gross difference that differentiates the mutation pattern of the chloroethyl ester from that of other alkyl-methanesulphonates.

Auerbach[2] suggests that the differential mutagenicity of the chloroethyl ester may be a function of the chloroethyl group which, she maintains, is known to be mutagenic. However, the fact that the chloroethyl group in 'mustards' (S- and N-mustards) is mutagenic does not mean that it is always so, irrespective of the configuration of the rest of the molecule. The biological activity of the chloroethyl group in 'N-mustards' has been shown by Ross[10] to be due to the tendency of these compounds to produce 'electrophilic' carbonium ions which would be expected to react with the 'nucleophilic' centres in the cell—particularly those of nucleic acids. Ross also demonstrated that the above tendency could be measured *in vitro* by the rate of hydrolysis of the chlorine atom under mild conditions. Since with 2-chloroethyl methanesulphonate, the chlorine atom does not hydrolyse in water at 37° C., whereas the methanesulphonoxy group does, there can scarcely be any doubt that the potential alkylating ability of the chloroethyl ester initially starts at the methanesulphonoxy end, as is the case with the other alkyl-methanesulphonates. If, however, the product of the initial reaction could be such as to 'activate' the chlorine atom of the chloroethyl group, then another alkylating centre would have been secondarily produced. Hence the search for the secondary agent that could account for the atypical mutagenic properties of the chloroethyl ester.

As clearly admitted in our article[8], the suggested formation of a secondary mutagen under the effect of 2-chloroethyl methanesulphonate was only inferential, and our idea about its chemical nature was purely speculative, but experiments were designed for testing our hypotheses. The first series of experiments using S-chloroethyl cysteine (one of the possible metabolites) has now been undertaken. The brood-mutation pattern for this compound was found to be of essentially the same type as that for 2-chloroethyl methanesulphonate, though indicating far greater selectivity for the early germ cells (details to be published elsewhere). An injected concentration of S-chloroethyl cysteine as low as $0{\cdot}4 \times 10^{-2}$ molar induces a sex-linked recessive lethal-rate of 30 per cent in the most sensitive spermatogonia as compared to a maximum of only 1·6 per cent among the sperm and spermatid stages. Other amino-acid mustards that are likely to be produced *in vivo* through the effect of the chloroethyl ester (particularly in conjunction with cysteine metabolism) will be tested for mutagenicity. However, the results with S-chloroethyl cysteine suggest that our reasoning as to the mode of mutagenic action of 2-chloroethyl methanesulphonate was on the correct lines: that the high response of spermatogonia under its effect is probably due to the production *in vivo* of a secondary mutagen other than the sulphonate itself.

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The editors of *Nature* have kindly given me leave to wind up this discussion with a few lines; they do not wish a continuation of technicalities, and I refer, therefore, for details of my criticism to my paper in *Z.i.A.V.* Here I only want to say this: although the reply by Drs. Fahmy seems to meet all my objections, it does in fact, on closer analysis, confirm that their methods and reasoning were, point for point, exactly as stated and criticized by me. Their reply contains no new references to published data; their insistence on the repeatability, in their experiments, of the relation between dose and effect is wholly irrelevant to the argument; the calculation in section 2 is based on too specious an argument to be taken seriously; finally, it is very difficult to understand why "an extraordinary efficiency in the production of visible mutations" by certain compounds should have been noted but not mentioned for a full year during which two lengthy interim reports came out, especially as this result was first made public in exactly the same kind of interim report. If the speculations on the action of *CB* 1506 have led to the discovery of a further interesting mutagen, they certainly have had heuristic value. It does not follow, however, that they were correct or even well founded. Two substances may well produce similar brood patterns without one being a metabolic descendant of the other (see the last section of my communication above). Moreover, it would not be the first time that an effective mutagen has been detected on the basis of a wrong or doubtful hypothesis.

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