CANCER

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VOLUME 6

PART X: CHEMOTHERAPY

PART XI: PUBLIC HEALTH AND NURSING ASPECTS OF CANCER

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PART X

CHEMOTHERAPY

CONTENTS

CHAPTER 1

THE EXPERIMENTAL BACKGROUND OF CHEMOTHERAPY

G. M. TIMMIS

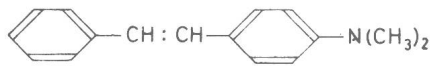
IN this chapter an account is given, mainly from the chemical point of view, of the action of chemicals on experimental neoplasms in mammals. The subject is confined to pure substances of defined chemical constitution and is divided into sections on miscellaneous agents, antibiotics, hormones, antimetabolites and biological alkylating agents. The experimental tumours used were transplanted in almost every case and frequently also they were transplanted into impure lines of mice or rats; nevertheless, results from different centres usually agreed and for the biological alkylating agents results on solid tumours ran parallel with the effects of the drugs on experimental leukaemias. The emphasis is laid on indicating structure–activity relationships where these are discernible and not on problems of experimental biological testing. These have been reviewed by Stock (1954, 1955), Foley (1956) and Hauschka and his colleagues (1956).

The use as test objects of human tumours transplanted in mice and rats (Toolan, 1953, 1954 and 1957) and into the cheek pouch of the hamster (Handler, 1956; Handler, Patt and Lutz, 1952; and Handler, Adams and Farber, 1957) has recently been introduced but so far only tests on a small number of compounds have been reported. The animals have to be treated with cortisone or with x-rays for the transplantation to be successful but after transplantation over several years they retain at least some of their original characteristic chromosome (Levan, 1956) and antigen composition (Korngold and Lipari, 1955).

Although the present lines of research may not lead to the curative treatment of cancer they may well provide valuable clues to the nature of the disease. Any evidence therefore that reveals a link between apparently different mechanisms of action would seem to be of particular interest. Evidence of this sort has been found in the investigation of the action of hormones and antimetabolites and there is some indication of a possible relation between the ways in which antimetabolites and alkylating agents work.

Until about 20 years ago numerous experiments, nearly always of a purely empirical nature, had been made over a great many years on the treatment of cancer with chemicals. The only result of any clinical value was the discovery that potassium arsenite (Fowler's solution) was useful in the treatment of chronic leukaemia (Lessauer, 1865). It is therefore not proposed to review this period in detail but to start a brief historical introduction with the now classical work of Haddow (1935, 1938), later developed in collaborative investigations (Haddow and Robinson, 1937, 1939; Badger and his colleagues, 1941–1942), in which it was shown that the polycyclic carcinogenic hydrocarbons were also tumour inhibitors. Since, following the work of Cook and his school, various structural features in these substances could be specifically associated with carcinogenic activity (Barry and his colleagues,

1935) there arose for the first time precise indications of how tumour inhibiting activity might be related to structure. Thus the chemist could begin to design structures on a rational basis with the hope of obtaining more active tumour inhibitors and eventually perhaps some chemotherapeutic success. Many compounds which inhibited the Walker tumour were found but such clinical trials as were possible were not promising. By reason of a structural relation to 1 : 2-benzanthracene (derivatives of which were tumour inhibiting), derivatives of triphenylethylene were examined. Further modification by the insertion of a dimethylamino group led to an active compound, and finally simplification of the structure yielded the highly active 4-dimethylaminostilbene (I) (Haddow and his colleagues, 1948a).



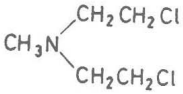
I

After investigation of a series of related structures the stilbene structure was combined with a $-N(CH_2CH_2Cl)_2$ residue by Haddow and his colleagues (1948*b*) to yield a derivative of nitrogen mustard (HN2) and thence led to a rather simpler class of aromatic mustards. In general, these compounds were far more active on the Walker tumour than any previously examined and this fact encouraged their active development. The trial of vitamin antagonists in experimental cancer appears to have grown, in part, out of an experiment by Maisin and his colleagues (1918) who found that yeast inhibited the incidence of benzpyrene induced tumours. Subsequently, Lewisohn and his colleagues (for a review of this work *see* Lewisohn, 1947) found that the folic acid present in yeast extract inhibited the growth of spontaneous mouse carcinoma. Subsequently a derivative of folic acid, pteroyltriglutamic acid, was claimed by Lewisohn to be similarly active but when this compound was tried by Farber and his colleagues (1947) on acute leukaemia in man the only effect was to exacerbate the disease. From these observations and experiments on induced folic acid deficiency in the rat (Farber, 1956) the idea of trying folic acid antagonists in the acute leukaemia of children was developed and led to the effective treatment of acute leukaemia and greatly encouraged pursuit of the antimetabolite approach.

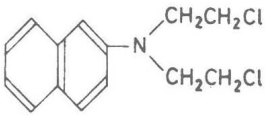
BIOLOGICAL ALKYLATING AGENTS

Drugs of this type were developed after the discovery that, following administration of N.N.di-2-chloroethylmethylamine or HN2 (II), severe depression of the haematopoietic system was caused. This effect, together with the toxicity of the drug towards rapidly proliferating cells and the induction of chromosome fragmentation, revealed a similarity to the effects of x-irradiation. This compound, which was a war gas, was first tested clinically during World War II under conditions of secrecy. The pharmacological effects have been reviewed extensively by Gilman and Philips (1946), by Boyland (1948) and by Philips (1950) and the examination for tumour inhibiting activity of HN2 and a large number of aliphatic derivatives was reported by Burchenal and his colleagues (1948) but without disclosing any

marked improvement over the original drug. The desire to lessen or remove the vesicant action and other toxic effects of HN2 led Haddow and his colleagues (1948*b*) to prepare and test analogues which bear an aromatic group in place of the methyl group, for example, III.

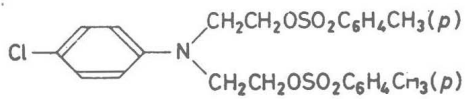


II

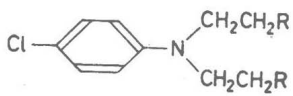


III

For the same reason, Haddow and Timmis (1951) prepared and tested a series of sulphonic acid esters (IV) which are chemically related to the nitrogen mustards in that the chlorine atom and the sulphonyloxy group, for example, $-OSO_2C_6H_4CH_3$ (*p*), both confer alkylating ability, that is, each ethane moiety is permitted to combine at a suitable site (nucleophilic centre), RH, to form, for example, V. Esters of acetic, trichloroacetic and benzoic acids corresponding to the sulphonic acid esters were also made and tested by Haddow and Timmis and found to be inactive as tumour inhibitors. The fact that this type of ester is both inactive and not an alkylating agent lent support to a conclusion suggested by the tumour inhibiting activity of HN2 and the aromatic mustards, namely that their common mechanism must be by alkylation.

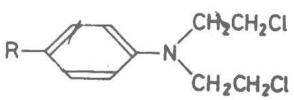


IV



V

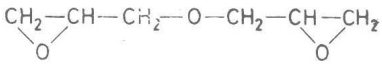
A number of aromatic nitrogen mustards (VI) were made by Ross (1953) which bore a variety of substituents in the benzene ring, for example, R = NH_2, CH_3O, Cl and NO_2. Taken in order these substituents vary from being strongly electron-releasing to strongly electron-attracting, and the corresponding mustards become



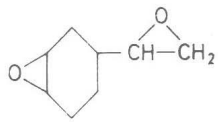
VI

progressively less active as alkylating agents and as tumour inhibitors. By these experiments the belief that the mustards act essentially as alkylating agents was confirmed and the alkylation was shown to be an SN1 type of action. Similar experiments in the series of sulphonic acid esters exemplified by IV again indicated an SN1 mechanism (Timmis, 1949) for the alkylation but the fact that IV and its unchlorinated analogue, unlike the mustards, cause no bone-marrow depression in experimental animals (Haddow and Timmis, 1951; Bushby and White, 1950) remains an interesting observation especially since the mechanism of alkylation is the same in both cases.

Other types of alkylating compounds containing two or three alkylating groups and showing the same biological effects as the mustards were found in the di-epoxides, for example, VII (Ross, 1950 ; Everett and his colleagues 1950) and VIII (Hendry and his colleagues, 1950) and ethyleneimine derivatives (*see* below)



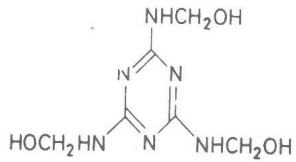
VII



VIII

and proof of the essential involvement of alkylating ability in the biological action was virtually complete. A prodigious number of mustards and ethyleneimine derivatives which have since been made and tested has provided further confirmation.

Tumour inhibiting activity was found by Hendry and his colleagues (1951*a*) in trimethylolmelamine (IX) but there was no clinical outcome of this work



IX

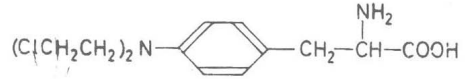
There is, however, some academic interest in it since the methylolamino group, $RNHCH_2OH$ is known only to alkylate amines, for example $R'NH_2$, to yield $RNHCH_2NHR'$, and this type of biological alkylating agent seems, therefore, to be set apart from the epoxides, mustards, ethyleneimines and sulphonic acid esters, since these can in addition alkylate anions, for example carboxylate or phosphate anions, and reaction of IX at such sites *in vivo* would be unlikely. It was conceivable that the action of the drug might have been due to the liberation of formaldehyde from the methylolamino groups, but a control experiment seemed to negate this hypothesis.

Of the four principal classes of alkylating agents, *i.e.*, the mustards, epoxides, ethyleneimine derivatives and sulphonic acid esters, the mustards and ethyleneimines have been the most extensively developed, and since these types and the sulphonic acid esters have provided drugs of some clinical value they are now dealt with in greater detail.

The mustards

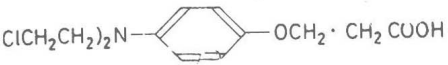
The most interesting products of the aromatic mustard series so far discovered have been *p*-(di-2-chloroethylamino)-phenylbutyric acid (chlorambucil), X, (Ross, 1953, *see* Everett and his colleagues, 1953) and *p*-(di-2-chloroethylamino)-L-phenylalamine, XI, (Bergel and Stock, 1954) known as Melphalan. The DL form of XII was independently discovered and tested by Larionov and his colleagues (1955), and is known as Sarcolysine.

X

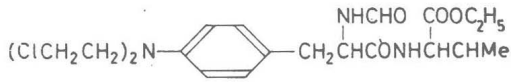


XI

Both compounds inhibit growth of the Walker tumour in low doses and are active lymphocyte depressors; they are being used clinically and X has over the past few years proved to be a useful drug in lymphocytic leukaemia, Hodgkin's disease and lymphosarcoma. An analogue of X, *p*-(di-chlorethylamino)-phenoxy-propronic acid (XII) (Davis and his colleagues, 1958) is even more active on the Walker tumour. The *laevo* (L) form of mustard XI is very much more active than the *dextro* (D) form and this difference may be explained by differences in the transport mechanism which depend upon the configuration of the drug molecule. Luck (1957) obtained transitory regression of a mouse melanoma wih the *laevo* but not the *dextro* compound. Peptide derivatives of XI, for example, XIII, have been

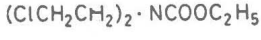


XII

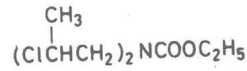


XIII

made and are active against mouse and rat tumours (Larionov and Sofina, 1957). A mustard derivative of alanine, $CH_3CH(COOH)N(CH_2CH_2Cl)_2$, was found by Izumi (1954) to be active on the Yoshida sarcoma. Mustards (XIV and XV) derived from the urethane structure were active on the Walker tumour but produced only a neglible effect on the bone marrow (Bushby and Murfitt, 1957). Investigation of a series of compounds derived from XIV and XV by substitution of the esterifying ethyl group by other alkyl groups led only to relatively inactive compounds. The active members are, however, intrinsically feeble alkylating agents and probably owe their activity to a metabolite.



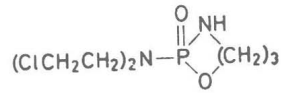
XIV



XV

Very recently a structurally related mustard (XVI) derived from carbamyl serine has also revealed tumour inhibiting activity accompanied by very little, if any, effect on the blood elements (Bergel and his colleagues, 1957). Another somewhat related mustard (XVII; Endoxan) was made with the thought in mind that certain phosphoramidases in which tumour tissue is particularly rich (Friedman and Seligman, 1954) might split the molecule to yield the nitrogen mustard, XVIII.

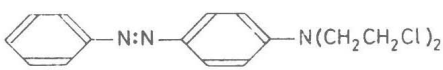
XVI



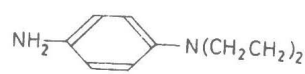
XVII

XVIII

A favourable therapeutic ratio and tumour regression in the treated animals was reported (Arnold and his colleagues, 1958). On preliminary clinical trial in a variety of types of cancer, however, good results have so far been seen mainly in cases of lymphosarcoma (Gross and Lambers, 1958). The possibility that XIV and XVI were perhaps active because XVIII was liberated by hydrolytic splitting led to the discovery that this compound, whose tumour inhibiting activity was first noted by Peczenik (1952) showed a sufficiently favourable therapeutic ratio in the inhibition of the Walker tumour (Davis and his colleagues, 1958) to merit clinical trial. The concept that mustards are in themselves very probably not selective for any particular type of cell has stimulated speculation as to how malignant tissue might be preferentially attacked. The problem has been discussed in general terms by Danielli (1952) and seems to be best approached by trying to design a mustard which would itself be relatively unreactive as an alkylating agent (and therefore unreactive biologically) in normal tissue, but by taking advantage of some abnormal factor in tumour tissue might be converted to an active mustard. An example of this idea has already been referred to in the case of XVII where the intrinsic alkylating ability of the $(ClCH_2CH_2)_2N$-grouping would be very low on account of the attached electron-attracting phosphoramide residue. The supposition that the abnormally high phosphoramidase activity of tumour tissue would lead to a preferential conversion of the inactive drug to the active mustard XVIII in tumour tissue, was, however, not borne out by the clinical results so far reported. Similar reasoning could be applied to the cases of compounds XIV and XV. If the enzyme required for their postulated splitting to XVIII was more active in tumour than in normal tissue some selectivity of growth inhibiting action could be expected. An analogous approach some years earlier by Ross and Warwick (1955) employed mustards of the type XIX.



XIX



XX

Reduction of the azo group ($R.N:N.R' \rightarrow RNH_2 + R'NH_2$) in various azo compounds is known to occur *in vivo* and it was hoped that this type of reaction would occur preferentially in tumour tissue and split the feebly reactive mustard of the type XIX to yield the highly active substance, XX. It was found that an enzyme system containing xanthine oxidase and a suitable hydrogen acceptor, that is, the sort of system which functions *in vivo*, did reduce XIX in the required way. By placing various substituents, such as methyl, carboxy, halogen and nitro, in the two benzene rings of XIX marked differences in reducibility of the azo group were caused and the ease of reduction in several cases varied roughly with the tumour inhibiting activity. There is thus good evidence that the postulated splitting occurs but as yet no evidence of a really selective action on tumour tissue. Examples of an unusual type of aromatic mustard (XXI) where the 2-chloroethyl groups were