# REVIEW ARTICLE

## ATTEMPTS AT CHEMOTHERAPY OF MALIGNANT DISEASES*

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### INTRODUCTION

IN 1953 a series of lectures was delivered at University College, London, on Some Chemical Aspects of Normal Growth[1], followed in 1954 by a review of abnormal growth and development of a non-malignant character[2]. As a logical sequel some chemical problems of malignant growth, or to use a more popular expression, of cancer and related diseases of the blood, are now considered. Even when most of the complex and vast biological issues are set aside, the theme remains formidable, and it is possible to touch on only certain aspects. The subject may be divided into three, (i) the chemistry of carcinogenesis and of agents causing malignant growth; (ii) the comparative chemistry of abnormal and normal tissues and cells, including the more dynamic aspects of metabolism; (iii) the chemistry applied to the preparation of compounds effective in the treatment of malignant diseases. The word chemistry is used here to embrace all aspects of the subject. Major points of (i) and (ii) were discussed recently during a lecture in the series "Scientific Basis of Medicine"[3].

Before commencing with (iii) a definition of malignant diseases should be attempted. This must include not only "cancer" or different forms of solid tumours, but also certain morbid states of blood-forming organs resulting in leukæmias and reticuloses. The higher organism which develops according to a genetically conditioned overall design may show outgrowths in the form of swellings, warts, polypi, papillomas and tumours. As long as these abnormalities of parts of organs, muscles, blood vessels, glands, skin, etc. are growing slowly and their enlargement stops at a certain stage or regresses, they are not endangering the whole organism, which is in some ways still in control of them, and they represent benign types of irregularities. However, if an outgrowth shows unrestricted growth potentialities, invades normal tissues, tends in its cells towards de-differentiation, irreversibility and autonomy, causes the formation of secondary metastases in other parts of the body and abnormal blood supply, and if the cells show abnormalities, lose their mutual adhesiveness, and are transplantable on an experimental basis, then one is confronted with one or other form of malignancy. This includes related irreversible diseases of the blood.

Benign and cancerous abnormalities can develop as a result of the impact on the living organism of a great number of primary agents[4].

* Condensed from a lecture given at University of London, University College, February 18, 1955.

While in the case of the benign types cessation of action of such agents and factors can lead to the arrest and final disappearance of the abnormality, most malignant states continue in the absence of any obvious stimulator, that is, they become "self-perpetuating". The puzzle of "self-perpetuation", the great variety of carcinogenic factors, the phenomenon discovered by Haddow[5], that carcinogens have carcinolytic properties, just as many drugs prepared as antitumour agents during recent years can produce neoplasms, have invited intensive work and even more intensive speculations about the mechanism of carcinogenesis. Only a summary of the possible cellular events in this pathological process can be given.

The hypothesis most generally accepted at present refers to somatic mutation of cells exposed to one or more endogenous or exogenous carcinogenic factors, and, in consequence, to a permanent change of metabolic powers. The adaptation hypothesis represents a variation on the theme of mutation and is based on work by Hinshelwood[6] on bacteria with their growth following the exponential law of autocatalysis, and on a preliminary report on immunological changes by Green[7]. The existence of viruses has been established in the case of Rous sarcoma in the fowl and Shope's papilloma in the rabbit but, in spite of many protagonists, has not been confirmed in other tumours. One day a reconciliation between these hypotheses might occur. Meanwhile, the search for chemical differences between normal and abnormal tissues continues. So far only quantitative differences have been found[8], particularly in water content, enzyme pattern, metal content, proteins, nucleic acids and phospholipids. Further analytical work is being intensively and extensively pursued.

## The Place of Antitumour Agents among Therapeutic Drugs

The word "chemotherapy" has been increasingly applied in recent years to the use of any drug whether acting pharmacodynamically, that is in a transient fashion on symptoms, pain, blood pressure, etc., or by replacement and restitution in the case of deficiencies, or by damaging and killing parasitical organisms. It seems better to follow Ehrlich's lead and limit the term chemotherapeutic agent to those preparations with antibacterial, antiprotozoal or antitumour effects, which are also known as "etiotropic drugs", that is, therapeutic substances acting on the cause of diseases. The role of the substances for restitutional therapy should be self-explanatory (enzymes and hormones in relation to cancer will be mentioned later[5].)

A consideration of how the modes of action of drugs used in infections and parasitical diseases are related to or differ from those which exert a transient pharmacodynamic effect, be it analgesic or sympathomimetic for example, may give some insight into the action of drugs which are capable of destroying tumour cells. The receptor hypothesis developed since Pasteur's days by Ehrlich, and by Cushny, Clark, Ing[9] and others has been generally accepted by pharmacologists and pharmacological chemists. Its concept is based on the idea that pharmacodynamic action is due to a combination of the drug with specific parts of the

cells in the responsive tissue, whether these parts are found in the cellular membrane or in the cytoplasm. Many experimental contributions strongly support this concept, for instance, the close similarity between the structure of the protagonist drugs, with their essential pharmacodynamic groups as Ing[9] called them, and their antagonists[10]. Little is known about the nature of these receptors apart from their being involved in membrane activities, controlling among other things the import and export of organic and inorganic material between extra- and intracellular regions. We think of them as enzyme systems.

The mode of interaction between drugs and enzymatic receptors could be postulated in a number of ways. These are, (i) by competition with normal coenzymes or substrates, (ii) by blocking active centres, or (iii) by interference with activators. One definite example of an enzyme acting as a receptor has been fully studied: true- and pseudo-cholinesterases have been shown[11] to interact stoichiometrically on specific spots of their protein surface with certain drugs either in a reversible or irreversible fashion. It should be remembered that of any enzyme which might be explored as a receptor of pharmacodynamically acting substances, the manner of such action, unless brought about with toxic doses (as opposed to effective doses) is transient, and takes place on responsive tissues, the cells of which are not necessarily actively dividing. In contrast the treatment with chemotherapeutic agents, aiming at neutralisation or death of the invading germs, must bring about more permanent changes. They are applied to growing and thus dividing cellular populations and their effects, whether produced on the basis of (i) competition for normal coenzymes and substrates (antimetabolites), (ii) blocking of active centres, (iii) interference with activators or through general damage, if not fully dangerous or deadly for the first generation will be passed on to the subsequent ones. The clinical efficacy of agents of this kind depends on the degree of difference between their toxicities to host and invader. As was pointed out by Albert[12], the greater the selectivity of the agent the more successful will be its interference with the cellular activities, growth and division of the parasitical organism.

*Antitumour agents and cellular constituents*

The agents influencing malignant growth should have much in common with antimicrobial drugs, at least as far as the so-called cytotoxic kind of antitumour substances is concerned, where interference with cellular activities is the target. There is, however, one great difference: the malignant cells stem, with the exception of those from experimentally transplanted tumours, from the host's own tissue and are often still closely related to it. They therefore show very much less selectivity towards toxic agents and antimetabolites in comparison with bacterial or protozoal cells. The margin becomes even narrower if one considers that the growth and dividing activities of malignant cells are shared by normal cells in those parts of the adult body which regenerate continuously, e.g., intestinal lining, bone marrow, skin, etc. The as yet only partial success of attempts to find cytotoxic agents with such a fine degree of selectivity is

one of the reasons why the chemotherapy of malignant diseases is still in its early infancy. Another reason is that there is not just a single disease called "cancer". Apart from the appearance of tumours in different tissues and their multi-etiology, each tumour of the same anatomical site may behave differently, particularly in man, and thus the discovery of "The Cancer Cure" is a near-impossibility.

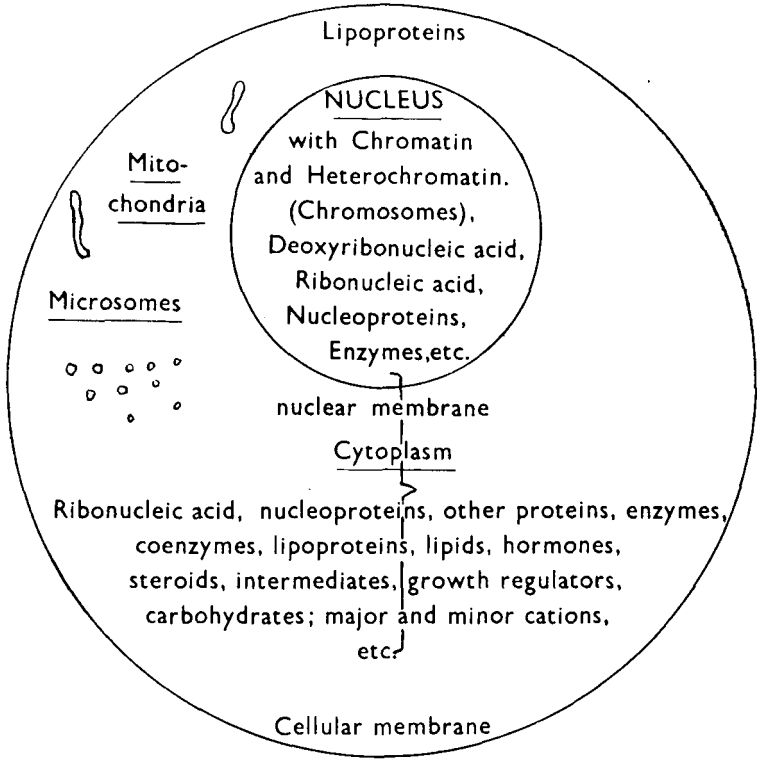
The part or full solution of the problem under discussion depends on finding substances which will either interact preferentially with some of the vital constituents of tumour cells, upsetting or destroying them by irreparably damaging the nuclear, cytoplasmic or membrane function, or, as such selectivity is difficult to establish, redress an imbalance of say hormonal or enzymatic activities. The idealised cell shown in Table I is surrounded by groups of substances which by chance or design have been found to produce some of the desired effects. The first are the so-called "biological alkylating agents" which react with certain cellular constituents in the cytoplasm or nucleus. Secondly, there are the antagonists and antimetabolites acting by inhibition of and competition with metabolic processes. Thirdly, there are some plant products which interfere with cell division. All act like a number of antimicrobial drugs, the initial damage caused by them being passed on to subsequent cell generations. Their efficacy rests on a very fine difference between the biological and chemical properties of normal and abnormal tissue. For instance, antagonists of coenzymes, such as deoxypyridoxine, have proved to be particularly effective experimentally if the tumours show low contents[13] in enzymes or coenzymes related to them; the underlying idea is that the antagonist knocks out completely the small amounts in the neoplasms but leaves the normal cell with its higher content relatively undamaged. Such mechanisms introduce into this field, governed largely by empiricism, an element of rationalism. Fourthly, an even more rational approach has been made by the introduction of hormone therapy[14] in cases of malignant disease of organs and glands under control of these biological regulators. This also represents an example of restitutional therapy, because by removing the pathological imbalance between œstrogens and androgens the existence of certain tumours, still dependent on such imbalance, is jeopardised. The fifth group represents another attempt at restitutional or replacement therapy, at present in its very preliminary stages, and is based on a working hypothesis of Haddow[15] and others[16] which aims to correct by means of enzymes or coenzymes a subtle deviation or deficiency in the growth controlling synthetic or degradative apparatus of the cancer cell as compared with that of a normal cell. The biological catalysts which regulate the catabolism of nucleic acids were first chosen for investigation as the acids are considered vital in the control of protein and nucleotide synthesis. Haddow[17] is at present experimenting with xanthine oxidase and Ledoux[18], working for the time being at the Chester Beatty Research Institute, and Brachet[19] are investigating ribonuclease. The results are promising enough to encourage further careful exploration of this approach. Radio-isotopes and X-rays form the sixth group.

## TABLE I

ANTITUMOUR AGENTS AND CELLULAR CONSTITUENTS

(1) *Biological Alkylating Agents*
(see Table II and Ross in *Advances in Cancer Research*, 1953, **1**, 397 and Haddow in *Physiopathology of Cancer*, 1953, 475).

(2) *Antagonists and Antimetabolites*
Antipurines: azaguanine, 6-mercaptopurine
Antifolics: aminopterin, amethopterin
Antiriboflavines: flavotin
Antipyridoxines: deoxypyridoxine
Anti-amino-acids: thienylalanine




(3) *Plant Products*
Bacterial: polysaccharides
Mould: actinomycin C, azaserine
Higher plants: podophyllotoxin, colchicine

(4) *Hormones*
Œstrogens and Androgens (see Burrows and Horning, Œstrogens and Neoplasia, 1952)
Cortisone, ACTH

(5) *Animal Products*
(Replacement(?) Xanthine oxidase (FAD, riboflavin), Ribonuclease, etc.

(6) *Ionising Radiations*
X-rays
Radio-isotopes

Table I groups antitumour agents around a diagram of an idealised cell showing the main constituents of its cytoplasm, microsomes, nucleus, chromatin (chromosomes) and membranes in the form of the macromolecules of ribo- and deoxyribonucleic acids, structural and functional proteins, hormones, carbohydrates, lipids and smaller molecules, such as metabolic intermediates, growth-regulators, trace metals.

*Cytoactive alkylating agents*

Even at this early stage in the chemotherapy of malignant diseases, the contributions by the chemists alone are so numerous that it is impossible to refer to them all in the course of this article. It is therefore proposed to concentrate on one group, namely the biological or cytoactive alkylating agents. This is appropriate as the Chester Beatty Research Institute during the last 7 or 8 years has done systematic work on these, and a fair share of the total results, so far available, have come from its laboratories[20].

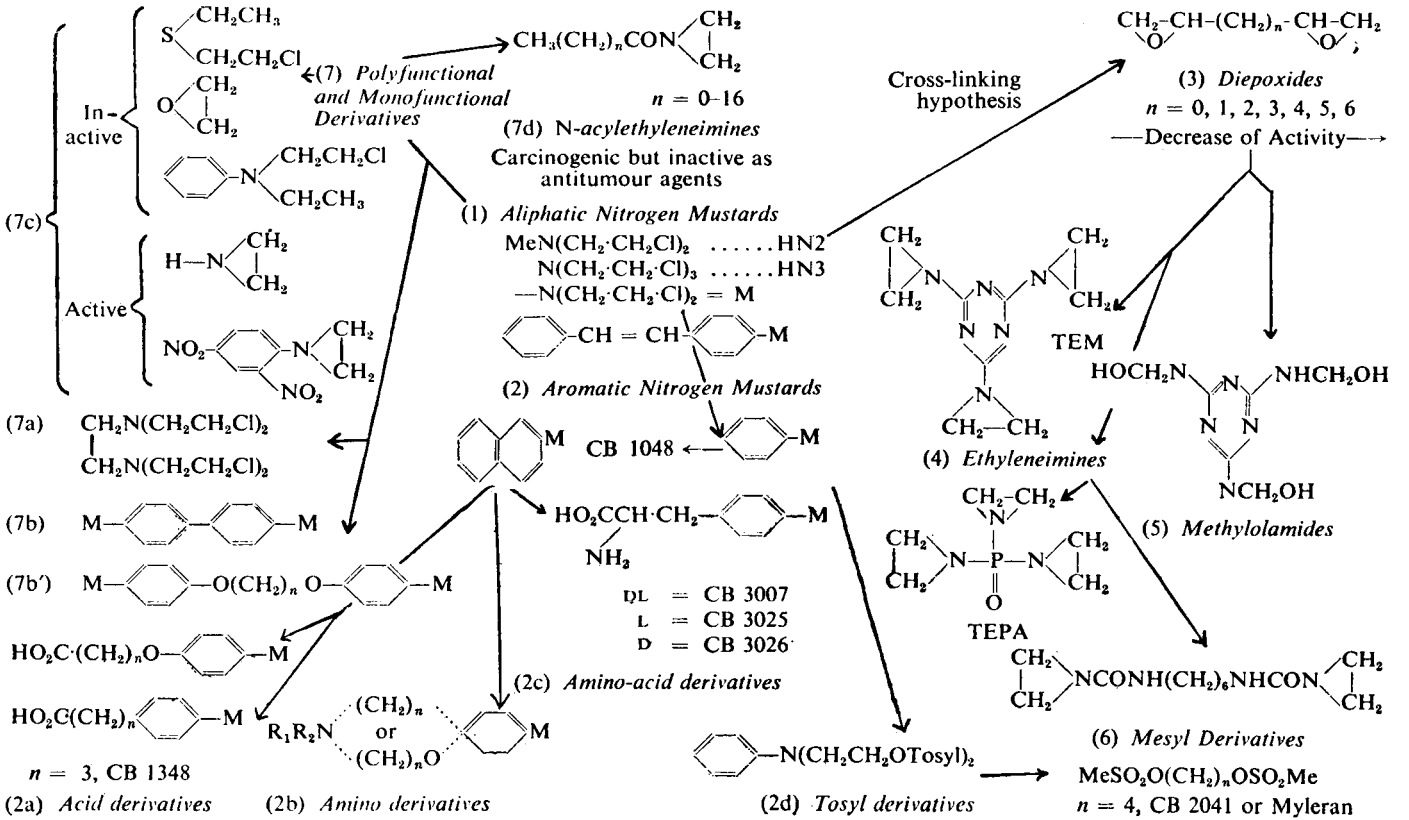
## Chemical Review

A chance discovery during war-time research on potential war gases[21] revealed the action of the aliphatic nitrogen mustards in lowering the blood leucocyte count. (Table II, sub-group (1).) It led to the use of di-(2-chloroethyl)methylamine, called HN2 and of the related tris-compound called HN3 in the treatment of malignant blood diseases, the leukæmias. As some of their experimental effects on blood (leucopænia), cells (chromosome damage), bacteria, flies, etc. (mutagenic action) were reminiscent of those produced by ionising radiations, such as X-rays, the name "radiomimetics" was proposed for them and for similarly acting drugs. However, their strong blistering action and instability made the search for other substances carrying the $N(CH_2 \cdot CH_2 \cdot Cl)_2 = M$ grouping, with better practical properties, very desirable. Haddow, Kon and Ross[22], following the important work by Haddow, Harris, Kon and Roe[23] on carcinogenic aminostilbenes, combined first the stilbene structure with M and then started a most fruitful systematic research into the chemistry and biology of the aromatic nitrogen-mustards, for example, CB 1048. (Table II, sub-group (2).) Desire for greater selectivity prompted Ross, Davis, Everett and Roberts[24,25] to prepare benzene derivatives which, in addition to the M-group, carried for instance, homologous fatty acid or $\omega$-hydroxy-fatty acid chains (sub-group (2a)). At the same time two other sub-groups were synthesised and studied: (2b) with cationic amino-alkyl residues[26] and (2c) with amino- and carboxyl-radicals combined, i.e., $\alpha$-amino-acids[27], which allowed the study of the configurational isomers. Again another team under Timmis explored substances in which the chlorine was replaced by sulphonoxy residues, such as toluene sulphonic or tosyl derivatives (2d)[28]. Attempts by Timmis at simplification led to a series of mesyloxy derivatives (sub-group (6))[29].

Another advance was achieved by independent workers subsequent to the publication of the cross-linking hypothesis by Goldacre, Loveless and Ross[30]. It had been observed that the mustards, including the old sulphur mustards, which carried at least two chloroalkyl groups (di- or poly-functional), were biologically more effective than those with one group (monofunctional). In an attempt to explain this fact, the three authors suggested that at a minimum, difunctional compounds were required for obtaining a cross-link between two or more macromolecules in the cell, such as proteins or nucleic acids (see Table I). As such cross-linking agents were known to be used in the textile industry, a number of research teams, following this working hypothesis, investigated, from an

## TABLE II

### CYTOACTIVE ALKYLATING AGENTS



antitumour point of view, compounds which had been applied to fibres, and they synthesised related ones. As a result epoxides (sub-group (3) )[31], ethyleneimines (sub-group (4) )[32,33,34], such as TEM, TEPA and a urea derivative and methylolamides (sub-group (5) )[35] such as trimethylolmelamine were tested and found to possess, like the halogen containing mustards, biological action on the basis of their alkylating properties. There remain some poly- and mono-functional compounds assembled as sub-group (7). While substances with more than two ethyleneimine groups have been mentioned under (4), so far only HN3 with more than the difunctional M-group has been quoted. Aliphatic derivatives, carrying two M (7a), do not possess enhanced activity[36]. On the contrary the aromatic derivatives (7b and 7b′ $n = 2$) prepared by Ross[37] and Everett[38] respectively were surprisingly devoid of activity. As previously indicated, most monofunctional substances, at least in comparable quantities, are biologically very much less effective than difunctional ones, but there are one or two exceptions as shown under (7c)[39].

Attention must be drawn to a most interesting discovery by Hendry *et al.*[49] with respect to a series of acyl-monoethyleneimines. While, practically, all cytoactive alkylating agents have proved to be carcinogenic in long-term experiments, the members of group (7d) prepared and investigated by the I.C.I. workers were found to be carcinogenic but not to show antitumour effects. This represents the first clear-cut exception to the reverse of the "Haddow phenomenon"[5], and raises the question of the mechanism of action of all these alkylating agents.

## Aspects of Physico-chemistry

The main points of the physico-chemical aspects of these agents are given below in Figure 1 (see also reference 20). For the purpose of this article they explain the meaning of "alkylating agents", particularly under biological conditions. The physico-chemists distinguish between two kinds of reactive substances, those that are electrophilic and which combine with electron-rich centres or negative particles (say $A^-$), and those that are nucleophilic (in some instances identical with negative particles) which combine with positively charged centres (in some instances identical with electrophilic substances). If a substance RYX divides, due to special circumstances, into the two ions $RY^+$ and $X^-$ the $RY^+$ ion can react very quickly with a negative $A^-$ forming RYA. This is called an $S_N1$ reaction-mechanism. On the other hand, a strongly combined RYX may in the presence of a powerful $A^-$ be forced into a dimolecular reaction, resulting in $RYA + X^-$; this reaction is following an $S_N2$-mechanism.

In the case of the biological alkylating agents it has been established that the nitrogen-mustards follow the former mechanism, and the mesyloxy compounds, for instance, the latter. While aliphatic nitrogen-mustards can produce via carbonium ions stabilised ethyleneimmonium ions still capable of further reaction, the aromatic nitrogen-mustards with their basically weaker nitrogen, react only as carbonium ions with their separated $Cl^-$ ions, or with $H_2O$ or with nucleophilic reagents ($A^-$).

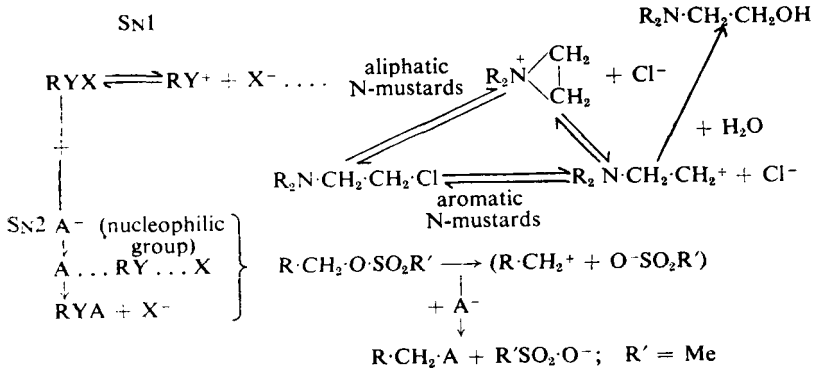



FIG. 1. Mechanism of action of biological alkylating agents.

It is of value to the chemist to know those groups in the molecules of protein and nucleic acids which are most likely to act as nucleophilic reactors. As shown in Table III, the ability to act is governed at a physiological *p*H (7·5) (i) by the $pK_a$ and therefore by the fraction $f$ of a group in a form likely to react, (ii) by the concentration $c$ of such a group in a biological system, and (iii) by the so-called competition factor $F_A$ (cf. Ogston[41,42] $= \frac{H_0 - H_a}{H_a A^-}$) which represents a comparison of the rates at which the nucleophilic group $A^-$ or water react with an electrophilic alkylating agent. Thus the probability of reaction under the conditions described is $F_A \times f \times c$. In practice (see Fig 2 below) only terminal undissociated $NH_2$ and dissociated $CO_2H$ groups and histidine in proteins, and phosphate groups and amino groups of pyrimidines and

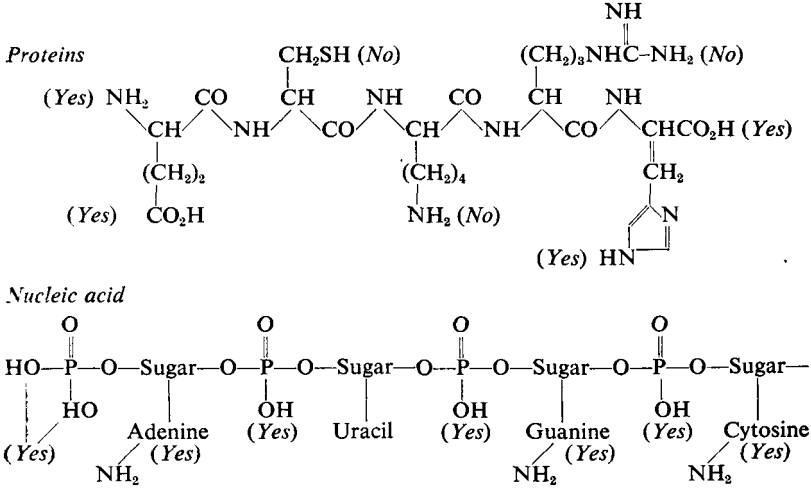



FIG. 2. Showing the groups which will react with an electrophilic alkylating agent. (After Ross.)

TABLE III

REACTIONS OF CARBONIUM IONS WITH ACIDIC AND BASIC GROUPS IN PROTEIN AND NUCLEIC ACIDS

(After Ross[20])

| "electrophilic" | "nucleophilic" | $pK_a$ | Fraction ($f$) of group in a form likely to react at $p$H 7·5 |
|---|---|---|---|
| $-CH_2^+$ | $+ {}^-OCR' \rightarrow -CH_2{\cdot}OCR'$ (terminal, aspartyl, glutamyl) | 3·0–4·7 | 0·9999–0·999 |
| $-CH_2^+$ | $+ {}^-O{\cdot}R' \rightarrow CH_2OR'$ (tyrosine) | 10·4 | 0·001 |
| $-CH_2^+$ | $+ {}^-SR' \rightarrow -CH_2SR'$ (cysteine) | 10·8 | 0·0005 |
| $-CH_2^+$ | $+ R'NH_2 \rightarrow CH_2NHR' + H^+$ (terminal α or ω) | 7·6–10·6 | 0·44–0·001 |
| $-CH_2^+$ | $+ R'NHC(=NH)-NH_2 \rightarrow -CH_2NHC(=NH)-NHR' + H^+$ (arginine) | 11·6–12·6 | 0·0001–0·00001 |
| $-CH_2^+$ | $+ R'$(histidine) $\rightarrow -CH_2N$(histidine)$R' + H^+$ | 5·6–7·0 | 0·99–0·76 |
| $-CH_2^+$ | $+ {}^-OP(=O)< \rightarrow -CH_2OP(=O)<$ (nucleic acid phosphate) | 2·0–6·0 | 0·9999–0·96 |
| $-CH_2^+$ | $+ R'NH_2 \rightarrow -CH_2NHR' + H^+$ (amino group of, cytosine, adenine, guanine) | 2·3–4·2 | 0·9999–0·999 |
| $-CH_2^+$ | $+ {}^-OR' \rightarrow -CH_2OR'$ (uracil, thymine OH) | 10·1–10·2 | 0·0025–0·002 |
| $-CH_2^+$ | $+ R'OH \rightarrow -CH_2OR' + H^+$ (pentose OH) | 13 | 0·00001 |
| $-CH_2^+$ | $+ HOH \rightarrow -CH_2OH + H^+$ | — | — |

There is no reaction with undissociated acidic groups or ammonium ions $RNH_3^+$.

purines in nucleic acids will be attacked by nitrogen-mustards, sulphonic acid derivatives, epoxides and ethyleneimines.

It is therefore permissible to say summarily that all the alkylating agents mentioned can be regarded as electrophilic reagents which will alkylate nucleophilic centres in the cell. Which of the interactions is the most likely to be responsible for the antitumour or carcinogenic effects cannot be answered with certainty (see Alexander on the reaction of carcinogens with macromolecules[43]). The writer personally prefers the view that interaction with nucleic acids is the cause of biological damage in that the breakdown or loss of shape of these vital molecules may profoundly upset the overall activities of cells (see Fig. 3).

Apart from other supporting evidence for this, for example the work by Butler *et al.*[44,45], recent *in vitro* results by Bullock[46] with the cholinesterases show that only relatively large concentrations of alkylating agents effect inhibition while experiments by Zamenhof[47] with a bacterial transforming

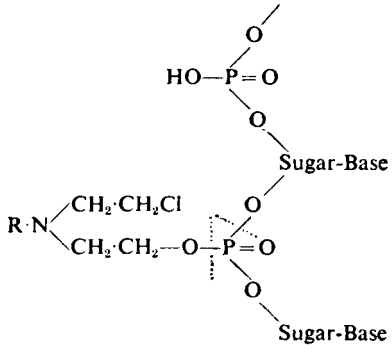



FIG. 3. Breakdown or alteration of shape of molecule.

factor DNA from *Hæmophilus influenzæ* demonstrate the great susceptibility of nucleic acids to the action of nitrogen-mustards.

## BIOLOGICAL EFFECT AND RESULTS

### *Biological effect*

It is pertinent at this point to indicate what is understood by biological effect in relation to antitumour agents. This is the response to the agent in a series of biological tests carried out to investigate its antitumour properties. The tests which are employed at the Chester Beatty Institute are not unlike those performed in other laboratories engaged upon similar work.[48] The pattern is as follows. A screening test is employed in which the transplanted Walker Carcinoma 256 is used. The fast growing tumour which in the form of fragments is implanted into the flank of the rat kills the animals after about 20 to 25 days when fully developed. An agent showing activity will either reduce the size of the tumours in comparison with untreated controls, thus prolonging life, or bring about their complete disappearance. A substance showing promise may then be submitted to further testing:—

i. In mice on various forms of ascites or liquid tumour cell suspensions, sarcomas and spontaneous mammary carcinomas.

ii. In rats on the resistant August tumour.

iii. For carcinogenic properties over a long period of administration.

iv. For its cytological effects on dividing cells in animal tumours or root tips.

v. For mutagenic effect on drosophila flies.

vi. For toxicity over a 3 to 4 week period.

vii. For effects on the weight and blood forming organs of the normal rat (with possible changes in erythrocytes and leucocytes).

The substance may then be submitted for clinical trial.

### *Biological results*

Ross and colleagues[20], who have been largely responsible for the physicochemical data presented, have also found that the tendency of alkylating agents, especially of aromatic nitrogen-mustards, to form carbonium ions and thus become reactive, can be expressed by their rates of hydrolysis.

The results with a great number of compounds carrying, in addition to M, activating (electron repelling) and deactivating (electron attracting) groups, indicate that a certain minimum reactivity is required for producing a biological effect. The hydrolytic rates and thus the reactivity of the series of substances synthesised by Ross, Davis, Everett and Roberts and mentioned before as aromatic mustards carrying in the *para*-position acid chains of varying length (see Table IV) are nearly all of the same order

TABLE IV

BIOLOGICAL PROPERTIES OF AROMATIC *N*-MUSTARD ACIDS

R = $Cl{\cdot}CH_2{\cdot}CH_2$, $Cl{\cdot}CH_2CH_2$ > N 

| Compound | Per cent. hydrolysis in 30 minutes in 1 : 1 acetone-water at 66° C. | Biological activity |
|---|---|---|
| I. R·COOH | 15 | ± |
| II. $R{\cdot}CH_2{\cdot}COOH$ | 39 | − + |
| III. $R{\cdot}CH_2{\cdot}CH_2{\cdot}COOH$ | 41 | + |
| IV. $R{\cdot}O{\cdot}CH_2{\cdot}COOH$ | 48 | − |
| V. $R{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}COOH$ | 42 | + + + |
| VI. $R{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}COOH$ | 52 | + − − |
| VII. $R{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}COOH$ | 39 | ± |
| VIII. $R{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}COOH$ | 50 | ± |
| IX. $R{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}COOH$ | 44 | + + |

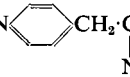
(After Haddow, Ross, Davis, Everett and Roberts.)

and cannot be correlated quantitatively with the effects on the Walker carcinoma. However, it became quite clear that the activity was strongly influenced by the number of atoms, whether C only or O and C, between the ring and the acidic COOH, reaching a maximum with the two compounds having 3 atoms. One, the *p*-M-phenylbutyric acid or CB 1348, is undergoing clinical trials.

TABLE V

BIOLOGICAL PROPERTIES OF PHENYLALANINE DERIVATIVES

DL = CB 3007, L = CB 3025, D = CB 3026

$(Cl{\cdot}CH_2{\cdot}CH_2)_2$ N  $CH_2{\cdot}CH{\cdot}COOH$ | $NH_2$

Weight (g.) of individual tumours (Walker rat carcinoma) at 13 days after implanation and 12 days after administration of the D-, DL- and L-forms of *p*-*N*-di (chloroethyl)-phenylalanine at a dose of 1 mg./kg. intraperitoneally in oil.

| Control | D- | DL- | L- |
|---|---|---|---|
| 115 | 44 | 15 | 0·5 |
| 90 | 34 | 5 | 0 |
| 81 | 30 | 4 | 0 |
| 39 | 28 | 3 | 0 |
| 37 | 23 | 1 | 0 |
| 31 | 20 | 0 | 0 |
| 30 | 19 | 0 | 0 |
| 28 | 13 | 0 | 0 |
| 22 | 10 | 0 | 0 |
| | 6 | 0 | 0 |

(After Bergel, Haddow and Stock[27].)

As all these substances have the same *p*-M-phenyl group and are water soluble as sodium salts, the differences in activities must be due to some property yet to be established, perhaps connected with transport to the cells or primary attachment there. In Table V quantitative data are given on the suppression of the Walker tumours by the L-, D- and DL-forms of the phenylalanine derivatives, from which it may be seen that using 1 mg./kg. of

bodyweight in the rat the L-isomer proved to be the most active compound. Thus the introduction of an α-amino group has enhanced the antitumour activity of the corresponding phenylpropionic acid derivative (Table IV, compound III) and the resolution of the racemic phenylalanine derivative has led to a synthetic compound which is among the first products showing selectivity of antitumour action on the basis of steric configuration. The only other example, reported from America[49], is azaserine, obtained from moulds, which as the L-isomer is more potent than its D-form. The reason for this selectivity is speculative, although pharmacodynamic drugs, such as adrenaline, are known to show this phenomenon. It is possible that the degree of initial attachment of the antitumour substances on an optically active cellular constituent or their penetration of the cell wall is governed by their configuration. Like CB 1348, the L-phenylalanine derivative CB 3025 is at present undergoing clinical trials.

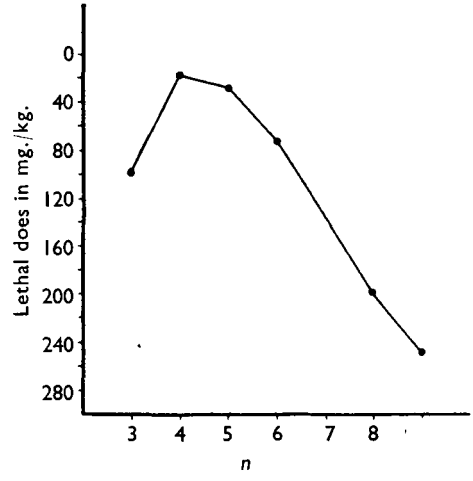



FIG. 4. Toxicity of compounds $MeSO_2 \cdot O \cdot (CH_2)_n \cdot O \cdot SO_2Me$. Dose producing fatal hæmorrahage (after Elson and Timmis).

Next, brief reference should be made to the biological effects of the dimesyloxyalkanes, synthesised by Timmis[29], which differ from the nitrogen-mustards as alkylating agents in that they react according to an $S_N2$ mechanism. In the homologous series, where the alkane bridge

TABLE VI

EFFECT OF SERIES $MeSO_2 \cdot O \cdot (CH_2)_n \cdot O \cdot SO_2Me$ ON CIRCULATING NEUTROPHILS IN THE RAT

| $n$ | Dose mg./kg. (D) | Per cent. fall in neutrophils from normal (F) | $\frac{F}{D}$ |
|---|---|---|---|
| 3 | 40 | 50 | 1·3 |
| 4 | 8 | 70 | 8·8 |
| 5 | 12 | 85 | 7·1 |
| 6 | 40 | 60 | 1·5 |
| 7 | 60 | 40 | 0·7 |
| 8 | 80 | 50 | 0·6 |
| 9 | 100 | 40 | 0·4 |

(After Elson and Timmis cf. ref. [29].)

varies from 3 to 9 carbons, toxicities show a maximum when $n = 4$ (Fig. 4). These compounds were found by Elson[cf. 29] (Table VI) to reduce, in the rat, the number of circulating neutrophils more than the number of

lymphocytes, and that again the compound with 4-carbons (CB 2041, Myleran) produced maximum effects. Figure 5 (see Elson[50]) compares the effects of Myleran on weight and blood picture with those of X-radiation and CB 1348. Interesting differences have been established: X-rays (400–500 r) produced a double fall in bodyweight and in white

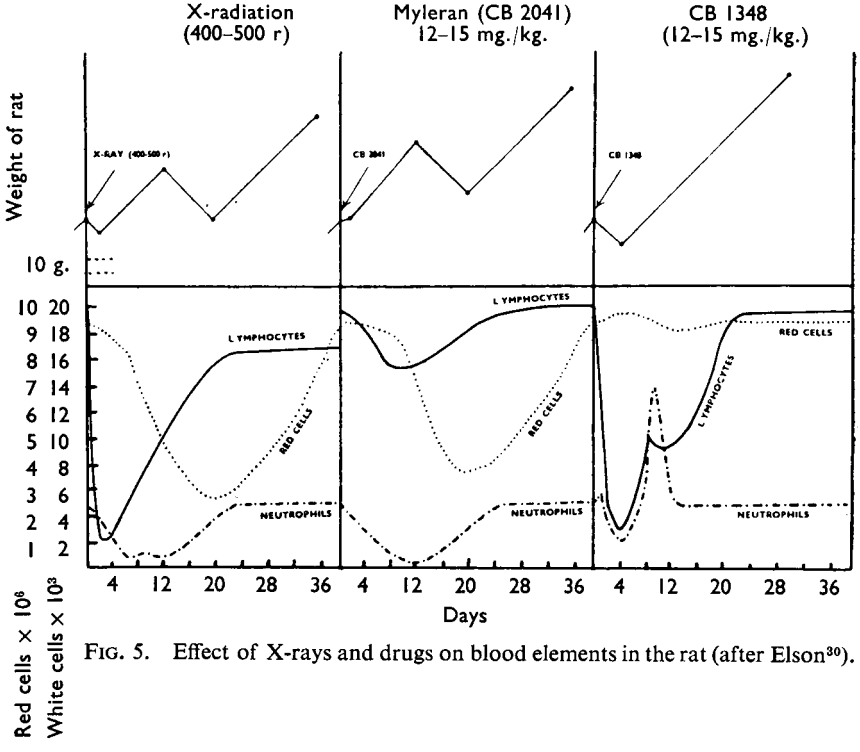



FIG. 5. Effect of X-rays and drugs on blood elements in the rat (after Elson[30]).

and red corpuscles, Myleran hardly influenced the level of lymphocytes, and CB 1348, while considerably lowering it, exerted only a transient effect on neutrophils. However, when Myleran and CB 1348 were administered together a result was obtained which approached that of radiation (Fig. 6; see Elson[50]).

Both X-radiation, by producing radicals, and alkylating agents share a number of biological properties, the most undesirable of which is the production of increasing resistance of the hæmopoietic organs to their action. However, in view of the differences demonstrated, the trivial name "radiomimetics" should be used with caution.

*Clinical investigations*

Turning to clinical investigations and once more to Myleran, its usefulness in cases of the myeloid form of leukæmia has been established beyond doubt[50]. Figure 7 shows the graphic story of one such case, as followed by Galton[51]. The abnormally high levels of neutrophils or

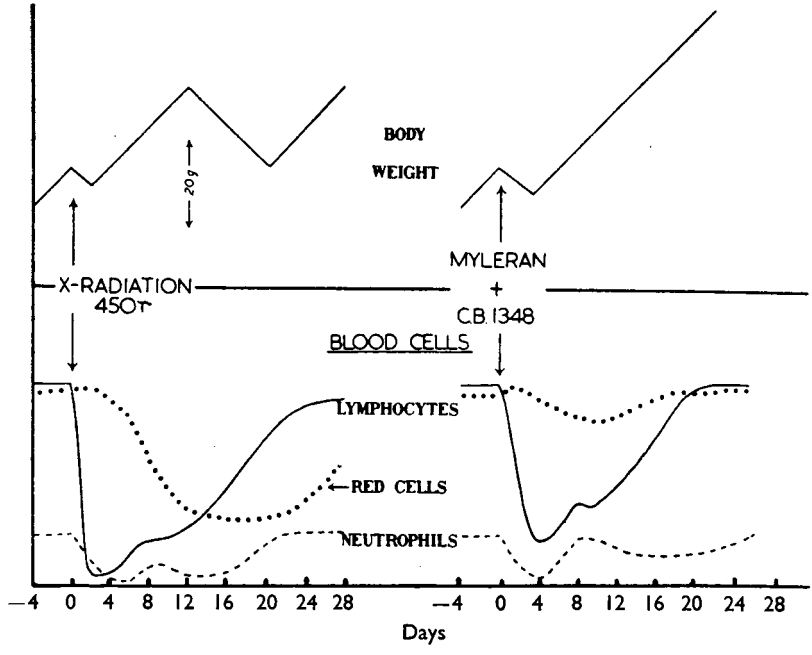



FIG. 6. Comparison of the effect on the blood picture of X-radiation and a combination of Myleran and CB 1348 (after Elson[50]).

the myeloid variety of white blood cells have been successfully brought back to near normal. It is too early yet to produce similar examples of lymphatic leukæmia, after the treatment with CB 1348 and 3025. To summarise: in Table VII[52] there are assembled those chemotherapeutic agents, previously mentioned, which have been and are used on human patients. It is true that with the exception of prostate and breast only

TABLE VII

DRUGS USED IN THE CLINICAL TREATMENT OF MALIGNANT DISEASES

| Drug | Type of disease influenced |
|---|---|
| Œstrogens | Cancer of prostate |
| Œstrogens | Cancer of breast |
| Androgens | Cancer of breast |
| Nitrogen Mustard TEM | Lymphadenoma |
| Myleran, Arsenite, Urethane | Myeloid leukæmia |
| CB 1348, CB 3025 | Lymphatic leukæmia? |
| ACTH and Cortisone | Acute leukæmia |
| Antifolic Acid Compounds | Acute leukæmia |
| Antipurines, e.g., 6-Mercaptopurine | Acute leukæmia |

(After Boyland.)

leukæmias are quoted, and counting lymphadenomas to the reticuloses, so far no solid tumours. This is disappointing. Yet the beginning of effective chemotherapy in this field is not very much older than one decade. Though the rate of advance cannot yet reach that in the treatment of infectious diseases, the prospects are far from being dull. The

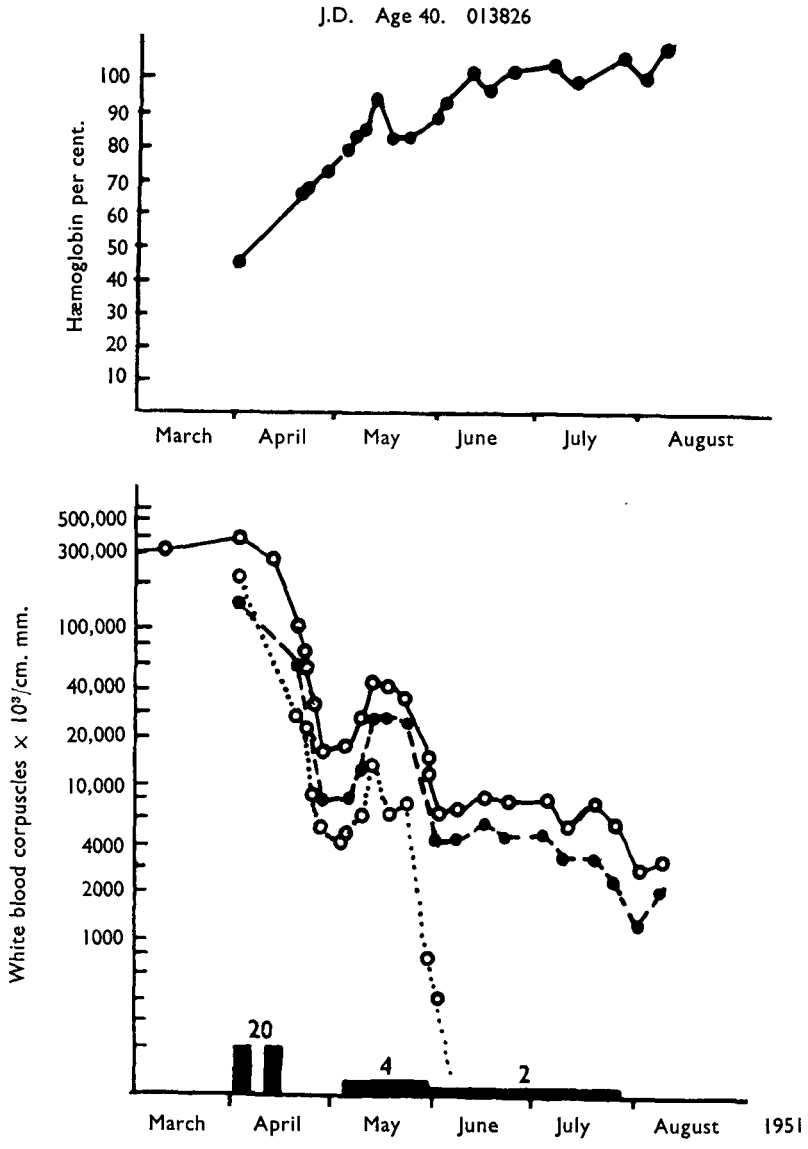



FIG. 7. Clinical effects of Myleran on a patient with leukæmia. The shaded area gives the dose of the drug in mg./day. ○—○ Total leucocyte count, ●- -● Neutrophils, ○ . . . ○ Immature forms (after Galton[51]).

use of drug combinations, each constituent possibly having a different mode of action, the systematic study of possible alterations and deficiencies in the aberrant cell and the subsequent attempts to make good such disastrous changes should lead in time to a wider and more decisive use of chemotherapeutics against more and more varieties of malignant diseases.

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