BRITISH MEDICAL JOURNAL

LONDON SATURDAY JANUARY 4 1958

THE CHEMOTHERAPY OF MALIGNANT DISEASE*

BY

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Sir Walter Langdon-Brown was my first teacher of medicine and my father's first teacher of physiology. He was, as many will recall, a physician remarkable for his erudition, his culture, and the breadth of his interests; nevertheless, the topic which I have chosen is not one with which he concerned himself. The very word "chemotherapy" was not in common usage during his professional life; the treatment of malignant disease was surgery, radiotherapy, and morphine; to have suggested that any other methods were worthy of respectful consideration would have been thought visionary by the profession at large and impertinent by the surgeons. There can be no doubt, however, that the advances the last few years have seen in the pharmacological attack on cancer would have fascinated one of the first to appreciate the importance of the chemical approach to clinical medicine and therapeutics.

This lecture is concerned with chemotherapy in its most exact sense, and the treatment of cancer with hormones and with radioactive isotopes is outside its scope. In it the terms "cancer" and "malignant disease" are used interchangeably to indicate proliferative lesions which display autonomous, continuous, and invasive growth. The proliferation is usually rapid and the constituent cells often show anaplasia, or an appearance which suggests, possibly incorrectly, that reversion to a more primitive type has taken place. This definition is broad enough to embrace not only such diseases as carcinoma of the breast and stomach but also leukaemia and even Hodgkin's disease. Current opinion demands their inclusion; cancer must now be defined in terms of dynamic cell behaviour and not by the static morphological criteria of the paraffin section.

Theoretical Considerations

Therapeutic advances are seldom the product of logical reasoning. They are more commonly the result of haphazard empiricism, often based on false assumptions. The use of salicylates in the treatment of rheumatic fever by Maclagan in 1876—a story Langdon-Brown enjoyed telling, for it appealed to his sense of the ironic—is the classic example of how progress in treatment is made. Maclagan believed that, in the orderly Victorian universe in which he lived, the natural remedy for each disease was to be found where that disease was prevalent. Noting the frequency both of rheumatic fever and of the willow tree in marshy country, he was led to use the salicin obtained from the second in the treatment of the first. After the first empirical leap in the dark, progress comes from working backwards, analysing the reasons for success, and building upon this more solid foundation. It will be seen that the chemotherapy of malignant disease is following this traditional pattern, and its theoretical basis is largely derived from a rationalization of its successes.

A rational treatment of cancer has for long seemed a forlorn hope, because the "cause" of the disease remains a mystery. Ablative surgery, where applicable, does not, of course, fall within the description of rational; although often the best that can be offered in the circumstances, it is always a tacit admission of failure. Much of the defeatism which surrounded this problem was due to aiming too high and to fixing attention on discovery of the ultimate cause. Ultimate causes, by definition, never can be found.

It has been customary when contemplating a malignant growth to think of it in terms derived from the study of inflammatory processes: to assume, that is to say, that it represents a reaction on the part of the tissues to some external agent. Reflection reveals no basis for this belief. Whatever may be the initiating cause, the hall-mark of cancer is an alteration in the reproductive behaviour of its constituent cells. Once this new pattern has been acquired it becomes a permanent characteristic. These activities depend upon the genes, and when the mutation which alters cellular reproduction in this fashion has taken place it will perpetuate itself. The cause of this genetic change may be evanescent, but the abnormal pattern of behaviour will have become irrevocably fixed and continuous invasive growth is the sequel. Control of the initiating cause may be necessary for the prevention of cancer, but for its cure a direct attack on the genes is required.

A theoretical approach to this problem would demand knowledge of how the gene transmits its behavioural characteristics. It has long been known that desoxyribose nucleic acid (D.N.A.) is the essential component of the chromosome, and it has been established that structurally different types of D.N.A. exist (Brown and Watson, 1953; Bendich *et al.*, 1953; Chargaff *et al.*, 1953). This demonstration has provided support for the belief that differences in genetic behaviour may depend upon differences in the chemical constitution of the genes. There is, moreover, indirect evidence that the D.N.A. metabolism of healthy and neoplastic cells differs. Malignant and analogous normal cells from the same animal have been grown side by side in tissue culture, and have been shown to differ in their susceptibility to substances known to interfere with nucleic acid metabolism (Biesele *et al.*, 1950). There is therefore some foundation for the view that the malignant behaviour of the cancer cell depends upon the chemical

*Being the Langdon-Brown Lecture delivered at the Royal College of Physicians of London on November 12, 1957.

structure of its genes, and that this is demonstrably different from that of analogous normal cells.

A parallel has been drawn between the cancer cell and an infecting bacterium (Rhoads, 1956). In a sense, the cancer cell fulfils Koch's postulates: it can always be demonstrated in the lesion in question; it can be cultivated outside the body; and when reinoculated into an animal it will reproduce the disease. A further similarity is to be found in the manner in which both bacteria and cancer cells develop resistance to chemotherapeutic agents initially able to restrain their growth. The analogy breaks down when pressed too far. The immune mechanisms, which are so important in resisting bacterial invasion, do not operate in malignant disease. The cancer cell is not "foreign" in the sense that this description may be applied to the bacterium. Nevertheless, the parallel provides a helpful basis for chemotherapeutic research.

The metabolism of bacteria differs greatly from that of their hosts, and one such difference is responsible for the therapeutic success of sulphanilamide. Investigation of its mode of action provided the invaluable concept of the antimetabolite (Woods, 1940). If the metabolism of cancer cells differs sufficiently from that of healthy tissues, it should be possible to find a substance which will block some metabolic pathway essential to the survival of the first, but unimportant to the second. For reasons already considered, it is logical to regard nucleic acid metabolism as the most promising target for attack, and there is some evidence that in this respect cancer cells and healthy cells may differ. There is therefore no theoretical reason why an agent which will exert a selective toxic action on the cancer cell should not be devised.

Such reflections are responsible for the hope and enthusiasm which now illuminate this problem. Research and the clinical trial of new chemotherapeutic agents proceed with ever-increasing momentum. Developments have been along two main lines. The first is largely empirical. Innumerable compounds, structurally related to those known to have some effect in malignant disease, have been synthesized, their action in selected animal tumours observed, and, if they appear to hold promise, clinical trial made after due pharmacological study. The second aims at the production of substances which may be expected to interfere with nucleic acid metabolism mainly by antimetabolic effect. These again are subjected to the same process of "screening." The labour and expense entailed before even one promising agent is discovered by these methods are immense and have limited this type of research to a few centres.

Any optimism which the tone of the earlier paragraphs may have aroused is sternly corrected by consideration of the results so far obtained in the treatment of human malignant disease. It has been said that the chemotherapy of cancer is in its "presulphonamide stage." It must be admitted that no patient has yet been cured by chemotherapy and that there are only certain tumours which show even a temporary response. These are, in the main, such systematized neoplastic disorders as leukaemia and reticulosarcoma, with occasional instances of other disseminated cancers. The selectivity of the agents at present available is but slight, and their toxic action is shown almost indifferently by malignant and by healthy cells. It is therefore only in tumours which present a wide front to their attack that any effect is to be expected. Moreover, the difficulties of clinical trials are considerable. No patient in whom cure, or even palliation, is likely to be obtained by conventional methods can justifiably be treated by chemotherapy. In consequence, therapeutic trials are limited to patients with leukaemia, reticulosarcoma, and Hodgkin's disease, and to those with inoperable and disseminated malignant disease.

The chemotherapeutic agents which have been shown to exert some beneficial effect upon human cancer fall into two broad groups. These are the cytotoxic drugs and the antimetabolites. The mode of action of the first is uncertain, and they are assumed to exert their effects directly upon the nucleic acids of cell nuclei. The general notion believed to underlie the action of the antimetabolites is now well recognized.

CYTOTOXIC DRUGS

The cytotoxic drugs in common use are certain alkylating agents and a heterogeneous collection of which the mode of action is little understood.

Alkylating Agents: 1. The Nitrogen Mustards

Foremost among the alkylating agents are the nitrogen mustards, amine derivatives of mustard gas containing two beta-chloroethyl groups linked, not by a sulphur atom as in the original compound, but by one of nitrogen. Their therapeutic use is a direct outcome of research into their systemic effects when used as a weapon of chemical warfare. There were, however, earlier indications which, seen in retrospect, might have pointed the way. Mustard gas was noted to exert a profound effect on the haemopoietic system as long ago as 1918 (Stewart, 1918; Krumbhaar, 1919) and to inhibit the appearance of the tumours which follow the repeated application of tar to the skin of the mouse (Berenblum, 1929). Indeed, attempts to treat carcinoma of the breast with it were made by Adair and Bagg (1931), but were abandoned because of difficulties of administration.

During 1942 and 1943 renewed study of the nitrogen mustards again revealed their ability to depress haemopoiesis, and led two groups of workers to investigate their effects upon patients with leukaemia and related disorders (Gilman and Philips, 1946; Wilkinson and Fletcher, 1947). The compounds used were the methyl-bis and the tris beta-chloroethylamine hydrochlorides. The second of these has now been discarded by most workers because of its undesirable side-effects; the first may be regarded as the prototype of the nitrogen mustards in clinical use to-day.

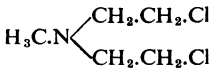
The activity of these substances, both against proliferating cells *in vivo* and in the test-tube, depends upon their reactive 2-chloroethyl radicals (Philips, 1950; Ross, 1953; Stock, 1954), and these properties are particularly evident in compounds possessing two or more such groups. In the body, or in solution, the nitrogen mustards undergo an intramolecular cyclization with the formation of ethyleneimonium compounds which are highly reactive on account of their alkylating capacity, and attack nucleo-proteins by combining with various of their component radicals such as carboxyl, phosphoryl, or amino groups (Ross, 1953).

Once it was appreciated that the cytotoxic effect of these drugs depended upon their 2-chloroethyl groups, a number of analogues were prepared in which the N-methyl group was replaced by other substituents. These alterations affect solubility and possibly distribution within the body, thus determining to some extent the tissue or cell type which will bear the brunt of the cytotoxic attack. A great number of these analogues have been synthesized, but few have proved to possess greater value than the original HN2. Those that deserve mention are R48, the beta-naphthyl compound; R151, or beta-naphthyl-di-(2-chloropropyl)amine; and CB1348, or chlorambucil, a phenylbutyric acid mustard. Russian workers claim good results with several chloroethylamines not in use in other countries; these include

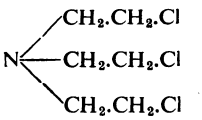
embichin, a methylamine mustard; novoembichin, a 2-chloropropyl derivative; dopan, a pyrimidine compound (Larionov, 1956); and sarcolysine (Larionov *et al.*, 1955), a phenylalanine mustard, an isomer of which had been synthesized independently in England. In Japan, an oxide of HN2, known as nitromin, has been used extensively (Ishidate *et al.*, 1951; Kimura *et al.*, 1952; Kurokawa, 1952). The varying solubilities of the different nitrogen mustards determine the doses which may be administered and the routes by which they may be given. There is little conclusive evidence that their activities against specific types of malignant disease differ in a way which cannot be accounted for by these factors.

Methyl bis-(beta-chloroethyl)amine hydrochloride, or HN2 (Fig. 1), has now been in use for 15 years (Goodman

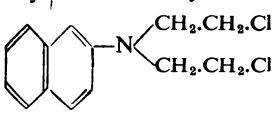
Methyl bis(beta-chloroethyl)amine hydrochloride (HN2).



Tris(beta-chloroethyl)amine hydrochloride (HN3).



Beta-naphthyl-di-2-chloroethylamine (R48).



Beta-naphthyl-di-(2-chloropropyl)amine (R151).

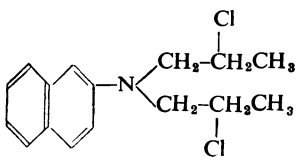


FIG. 1.—Nitrogen mustards.

et al., 1946; Wilkinson and Fletcher, 1947), and its value and limitations are well established. Its solubility necessitates its intravenous administration; injection has to be made as soon as the drug is dissolved, because of the rapid changes it undergoes in solution. The usual dosage is 0.1 mg. per kg. body weight repeated on four successive days. Nausea, vomiting, and anorexia are common three hours after injection and may last 24 hours or longer. Personal experience has shown that the whole dose of 0.4 mg. per kg. may be given at one injection without more severe disturbance than occasioned by each smaller dose; this method is now adopted as a routine. Its effect on the bone marrow is shown by a fall in the leucocyte and platelet counts, which reach their lowest levels about the eighteenth day and recover within four weeks. A second dose should not be administered for six weeks.

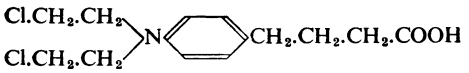
Its clinical applications have been reported by many workers (Gilman and Philips, 1946; Goodman *et al.*, 1946; Jacobson *et al.*, 1946; Karnofsky *et al.*, 1947; Wilkinson and Fletcher, 1946; ApThomas and Cullumbine, 1947; Wintrobe *et al.*, 1947). It has a clearly defined place in the treatment of Hodgkin's disease, particularly in the generalized stage of the disorder when systemic symptoms are prominent; improvement is, however, usually short-lived, seldom lasting more than eight weeks. It will induce remission in chronic myeloid leukaemia, but in general is less satisfactory as a method of control than many others. In chronic lymphatic leukaemia and reticulosarcoma the results are variable and less good than in Hodgkin's disease. Improvement has been noted in bronchial carcinoma, occasionally in disseminated carcinoma of the breast, and sometimes in carcinoma of the ovary.

R48 (erysan, chloronaftina) (Fig. 1) was introduced by Haddow *et al.* (1948). Several clinical trials have been reported (Matthews, 1950; Galton, 1951; Iverson and Meulengracht, 1951; Gardikas and Wilkinson, 1951; Videbaek and Kaae, 1954). Remission has been obtained in Hodgkin's disease, chronic myeloid and lymphatic leukaemia, and polycythaemia vera. It has the advantage of oral administration, but most workers agree that it is in no way superior to HN2, and that myelotoxic effects are more severe.

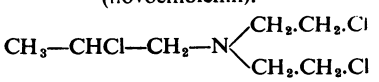
R151 (Fig. 1) was synthesized by Everett and Ross (1949). Good results were reported when used in combination with urethane in myelomatosis (Innes and Rider, 1955). Personal experience does not support these claims, and there are no data concerning its use alone in other malignant diseases.

CB1348, or chlorambucil (Fig. 2), was prepared by Everett *et al.* (1953) and proved effective against the Walker

p-di(2-chloroethylamino)phenylbutyric acid (CB1348).



2-chloropropyl-di(2-chloroethyl)amine hydrochloride (novoembichin).



2 : 6-dioxy-4-methyl-5-(2-chloroethyl)aminopyrimidine (dopan).

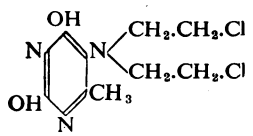


FIG. 2.—Nitrogen mustards.

rat carcinoma (Haddow, 1952). It is given orally in doses of 0.1–0.2 mg. per kg. for periods of four to six weeks. Extensive clinical trials have been reported (Galton *et al.*, 1955; Bouroncle *et al.*, 1956; Ultmann *et al.*, 1956; Farber *et al.*, 1956; Bernard *et al.*, 1956; Hansen, 1957). Its particular value is in the treatment of chronic lymphatic leukaemia, in which remissions are claimed by some observers in as high a proportion as 70%, although personal experience would regard this figure as optimistic. Similar results have been obtained in lymphoid follicular reticulosis, but the results in Hodgkin's disease and reticulosarcoma are less good than with HN2. It has the advantage of rarely causing gastro-intestinal upset.

Embichin, Novoembichin, and Dopan (Fig. 2) are products of Russian workers (Larionov, 1955). Excellent results are reported of their effects in Hodgkin's disease. *Sarcolysine* (Fig. 3) has also been synthesized by this group,

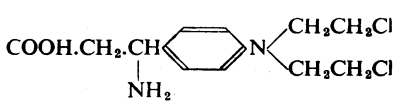


FIG. 3.—Di-2-chloroethyl-*p*-aminophenylalanine (sarcolysine, CB3025, melphalan)

and has proved valuable in their hands in disseminated seminoma and in hepatoma (Larionov *et al.*, 1955; Perevodchikova, 1956; Larionov, 1956). The laevo-isomer of this substance, CB3025, or melphalan, has been used in this country with results which have yet to be assessed.

Nitromin (Fig. 4), an oxidation product of HN2, was first prepared by Stahmann and Bergmann (1946). It has been used extensively in the treatment of malignant disease in

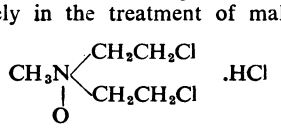


FIG. 4.—Methyl-*bis*(beta-chloroethyl)amine-N-oxide hydrochloride (nitromin).

Japan (Ishidate *et al.*, 1951 ; Kimura *et al.*, 1952 ; Kurokawa, 1952). Its effects are similar to those of HN2, but toxic reactions are said to be fewer. Benefit is reported in Hodgkin's disease and temporary improvement in rhabdomyosarcoma, neuroblastoma (Farber *et al.*, 1956), and occasionally in disseminated carcinoma (Stoll, 1956).

A final compound deserving mention is *hemisulphur mustard* (Fig. 5), a derivative of mustard gas itself, and

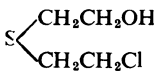


FIG. 5.—2-Chloro-2'-hydroxydiethylsulphide (hemisulphur mustard).

interesting in that it contains only one alkylating group. It has been used in advanced carcinoma (Seligman *et al.*, 1952 ; Rutenburg and Seligman, 1956). Side-effects are severe, but about 50% of patients showed significant benefit ; the response was particularly favourable in those with carcinoma of the ovary.

2. The Ethyleneimines

The observation that nitrogen mustards rapidly underwent changes in solution with the formation of ethyleneimonium compounds led to the investigation of other substances of related chemical structure.

The first of these was *triethylene melamine* (tretamine, or T.E.M.) (Fig. 6), a compound long in use in the textile trade

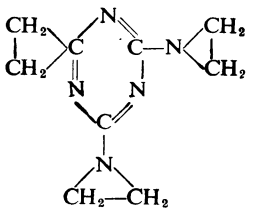


FIG. 6.—Triethylene melamine, tretamine (T.E.M.).

for improving the finish of rayon fabrics. Pharmacological and animal studies showed it to possess anti-neoplastic activity (Philips and Thiersch, 1950). Reports on its therapeutic value in human malignant disease rapidly followed (Wright *et al.*, 1950 ; Karnofsky *et al.*, 1951 ; Hansen and Bichel, 1951 ; Rundles and Barton, 1952 ; Kravitz *et al.*, 1952 ; Bayrd *et al.*, 1952 ; Silverberg and Dameshek, 1952 ; Paterson *et al.*, 1953 ; Axelrod *et al.*, 1953 ; Blackburn and King, 1954 ; Hambly and Robertson, 1955). Although originally given by intravenous injection, T.E.M. is now usually administered orally. Dosage varies ; a method which has proved safe is to give 2.5 mg. daily for two or three days and repeat the dose in a week if the leucocyte and platelet counts have not fallen significantly. Further dosage is regulated by changes in the blood picture. The average amount required is 35 mg. over a period of six weeks or less. It is administered fasting and followed by an alkaline draught.

Results similar to those given by HN2 are obtained in Hodgkin's disease and chronic myeloid leukaemia, but in chronic lymphatic leukaemia and lymphosarcoma they are somewhat better. Improvement has been noted in mycosis fungoides, carcinoma of the ovary, and lymphoepithelioma of the nasopharynx. In combination with irradiation, recession has been reported in retinoblastoma (Reese *et al.*, 1955).

A number of related substances, the *phosphoramides*, have been prepared. Those subjected to clinical trial are the triethylene, the diethyl-diethylene, the 3-oxypentamethylene, and the triethylenethio compounds, known respectively by

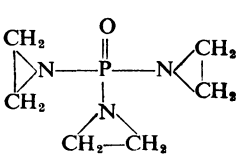


FIG. 7.—Triethylenephosphoramide (T.E.P.A.)

the code names T.E.P.A., D.E.P.A., O.D.E.P.A., and thio-T.E.P.A. The actions of all are similar, but D.E.P.A. and O.D.E.P.A. have been discarded as too unstable and toxic. With T.E.P.A. (Fig. 7) clinical benefit was noted in Hodgkin's disease, in neuroblastoma, and in several patients with disseminated melanoma (Farber *et al.*, 1953 ; Sykes *et al.*, 1953). It has been displaced now by thio-T.E.P.A. (Fig. 8), which is more stable and has a similar range of

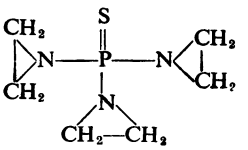


FIG. 8.—Triethylene thiophosphoramide (thio-T.E.P.A.).

action (Shay *et al.*, 1953 ; Leonard *et al.*, 1956), in addition to having some effect in carcinoma of the breast and ovary (Shay and Sun, 1955 ; Bateman and Carlton, 1956 ; Bateman and Larsen, 1956). Thio-T.E.P.A. is given by mouth in doses of 5–10 mg. daily until clinical improvement or evidence of bone-marrow depression appears.

A related sulphonamide, B.E.P. (Fig. 9) (Hendry *et al.*, 1951), has been found to provoke remission in a proportion

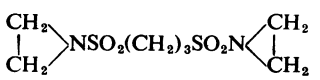


FIG. 9.—1,3-*bis*(ethyleneiminosulphonyl)propane (B.E.P.).

of patients with chronic leukaemia (Paterson and Kunkler, 1954). More recently, ethyleneimino-benzoquinone has been used in malignant disease (Wolf and Gerlich, 1956), and useful results are claimed in chronic lymphatic leukaemia and reticulosarcoma (Bernard *et al.*, 1957).

3. The Methanesulphonyloxy Alkanes

The observation that certain of the methanesulphonyloxy alkanes possessed the power of biological alkylation (Haddow and Timmis, 1951) led to the study of this group of compounds, one of which, *busulphan* (" myleran ") (Fig. 10) (Haddow and Timis, 1953), was noted to have a

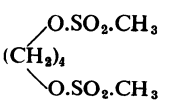


FIG. 10.—Dimethanesulphonyloxybutane (busulphan).

selective action upon the granular leucocytes of the experimental animal (Elson, 1955). Later, clinical trials proved it effective in chronic myeloid leukaemia (Galton and Till, 1955), although inactive against almost all other neoplasms. Subsequent reports have fully confirmed these claims (Consoli and Napoli, 1955 ; Haut *et al.*, 1955 ; Pribilla and Stollberg, 1955 ; Soares and Valente, 1955 ; Blackburn *et al.*, 1956 ; Galton, 1956 ; Louis *et al.*, 1956 ; Schilling and Meyer, 1956). The drug is administered by mouth. The usual dosage is 0.06 mg. per kg. daily. The blood picture returns to normal over four to eight weeks and the spleen shrinks. Maintenance treatment is continued at a level which will preserve a normal platelet count and not depress the leucocytes below 10,000 per c.mm. Remissions have been maintained for upwards of three years. Toxic side-effects are almost unknown, but, unless control is scrupulous, myeloid aplasia may occur.

The octane (Galton, 1956) and the nonane (Sykes *et al.*, 1956) analogues of busulphan have been submitted to trial, but have proved less active and more toxic.

Other Cytotoxic Agents

1. Urethane, or ethylcarbamate, long known to retard the growth of seedlings (Lefèvre, 1939), was later found to inhibit mammary carcinoma in mice (Haddow and Sexton, 1946). Clinical trials proved it an agent capable of inducing remission in chronic myeloid leukaemia (Paterson *et al.*,

1946 ; Cooper and Watkins, 1950) and of occasional benefit in prostatic carcinoma (Huggins *et al.*, 1947). It is used little now except in myelomatosis, either alone (Alwall, 1947 ; Rundles *et al.*, 1949) or in combination with R151 (Innes and Rider, 1955). In this disease its effects are difficult to assess, but seldom appear impressive.

2. *Colchicine Derivatives.—Demecolcine* or *deacetylmethylcolchicine,* an alkaloid derived from extracts of *Colchicum autumnale* (Santavy and Reichstein, 1950), and a related product, *N-deacetylthiocolchicine* (Velluz and Muller, 1954) are both capable of causing remission in chronic myeloid leukaemia (Moeschlin *et al.*, 1953 ; Leonard and Wilkinson, 1955). The second is said to be useful in combination with irradiation in the treatment of carcinoma (Huguenin *et al.*, 1955). In my hands demecolcine has been a difficult drug to manage, and its frequent toxic side-effects have led me to abandon it. Other workers endorse this view (Bousser and Christol, 1955).

3. *The Actinomycins.*—An antibiotic known as actinomycin A was isolated from cultures of *Streptomyces antibioticus* as long ago as 1940 (Waksman and Woodruff, 1940), but proved too toxic for therapeutic use. Actinomycin C, a product of *Streptomyces chrysomallus* (Brockmann and Grubhofer, 1949), has undergone extensive clinical trials in France and elsewhere (Croizat and Lacoste, 1955 ; Olmer, 1955 ; Tapie, 1955 ; Gilbert and Thommen, 1955 ; Ravina and Pestel, 1955). Cytopenia and gastro-intestinal disturbances are common results of its administration, and, although transient remissions may occur in Hodgkin's disease, it is less effective than HN2. Actinomycin D has been investigated and some promising results have been reported (Farber, 1955). It is possible that the actinomycins produce their effects by antimetabolic action.

THE ANTIMETABOLITES

The notion of the antimetabolite is derived from studies of the ability of sulphonamide to inhibit bacterial growth (Woods, 1940). Sulphanilamide bears a close chemical resemblance to *para*-aminobenzoic acid, a nutriment essential for the survival of the streptococcus ; by virtue of this similarity it is able to compete with it as a substrate in the enzymatic processes of bacterial metabolism. Because of its higher concentration, sulphanilamide is the successful competitor, but, because it is not identical with the normal substrate, the enzyme reaction cannot proceed to completion and the metabolic pathway is blocked. This concept of competitive inhibition of enzyme reactions has been widely exploited in the chemotherapy of cancer.

The use of antimetabolites in this connexion sprang from two lines of research. A folic acid analogue which had led to regression of mammary carcinoma in mice was unexpectedly found to accelerate the leukaemia process in man (Farber *et al.*, 1947). This observation suggested that folic acid deficiency might have the reverse effect and stimulated the synthesis and study of the folic acid antagonists. The other group of compounds which have established their therapeutic value are the antipurine drugs, and they are the products of a planned study of possible antagonists to the precursors of nucleic acid (Hitchings *et al.*, 1945 ; Hitchings and Elion, 1954).

Other antimetabolites, aimed at blocking enzyme reactions at different levels in the complex process of nucleic acid synthesis, have been devised ; these include pyrimidine, glutamine, amino-acid, and nicotinamide antagonists, but their worth as therapeutic agents is slight.

Folic Acid Antagonists

There are five groups of compounds which exert an effect antagonistic to folic, or pteroylglutamic, acid (P.G.A.). The first to be introduced were *4-hydroxyl derivatives* of P.G.A. (Martin *et al.*, 1947). Their antagonistic action is weak and they proved to have little therapeutic effect in leukaemia (Heinle, 1950). The *4-amino derivatives,* however, were rapidly found to be potent agents (Farber *et al.*, 1948), and representatives of this group have an established place in treatment. These drugs prevent the utilization of folinic acid, or citrovorum factor, as well as inhibit its formation from P.G.A. Those in common use are aminopterin (Fig. 11) and methotrexate or amethopterin (Fig. 12) ; at

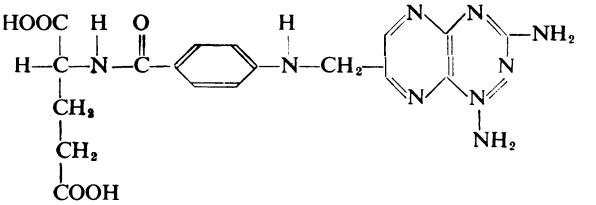


FIG. 11.—Aminopterin (4-aminopteroylglutamic acid).

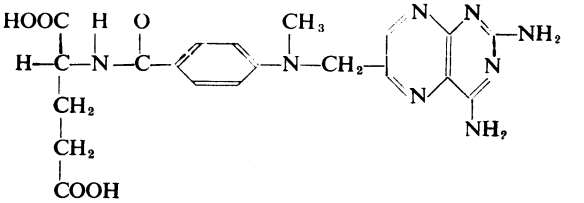


FIG. 12.—Amethopterin, methotrexate (4-amino-N^{10}-methylpteroylglutamic acid).

least four others have been submitted to clinical trial, but in general they offer no advantages over the first two.

Their place in therapeutics lies in the treatment of acute leukaemia in children. Benefit is rare in adults, but in the lymphoblastic type of the disease, and in patients below the age of 15 years, remission is procured in 60–70% (Burchenal, 1952 ; Fountain, 1954). Some objective effect has been noted in many other forms of cancer, such as rhabdomyosarcoma, Hodgkin's disease, lymphosarcoma, neuroblastoma, and chronic lymphatic leukaemia (Farber *et al.*, 1956).

Aminopterin and methotrexate are given by mouth, the first in daily doses of 0.5–3 mg., and the second of 2.5–10 mg. Toxic effects are common and include ulcerative stomatitis, diarrhoea, vomiting, alopecia, and gastro-intestinal haemorrhage.

The other classes of folic acid antagonist have little therapeutic importance. The *2:4-diaminopteridines* (Daniel *et al.*, 1947) have not been submitted to clinical trial. The *diaminodichlorophenyl pyrimidines,* of which D.D.M.P. (Fig. 13) (Hitchings *et al.*, 1952) is a representative, act in

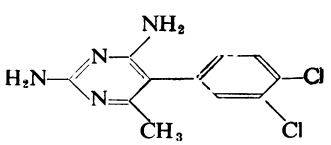


FIG. 13.—2.4-Diamino-5-(3′,4′-dichlorophenyl)-6-methylpyrimidine (D.D.M.P.).

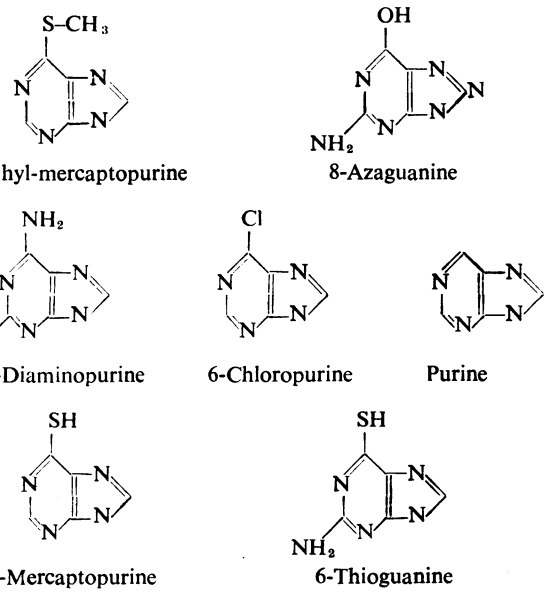
much the same fashion as the 4-amino derivatives of P.G.A., although probably at a different level. They are too toxic for therapeutic use (Murphy *et al.*, 1954). A closely related drug, *diaprim,* originally introduced as an antimalarial, has been found useful in the control of polycythaemia vera (Frost and Jones, 1954). The *dihydrotriazines,* also first synthesized in the search for antimalarials (Carrington *et al.*, 1951 ; Modest *et al.*, 1952), have not established their clinical utility (Farber *et al.*, 1956).

The ultimate appearance of drug resistance in leukaemia, at first responding to folic acid antagonists, is invariable ; when the disease becomes refractory to methotrexate, cross-resistance to all other folic antagonists, including D.D.M.P., develops (Murphy *et al.*, 1954).

Purine Antagonists

The purine antagonists (Fig. 14) are the fruits of a planned investigation into antimetabolites which might

impede nucleic acid synthesis. The first submitted to clinical trial was *2 : 6-diaminopurine*, or D.A.P. (Hitchings *et al.*, 1948), but it proved disappointing (Burchenal *et al.*, 1951), and its place was rapidly taken by 6-mercaptopurine, or 6-M.P. (Elion *et al.*, 1952). Clinical studies with this drug (Burchenal *et al.*, 1953) showed it capable of inducing remission in acute leukaemia and in chronic myeloid leukaemia ;



6-Methyl-mercaptopurine — 8-Azaguanine

2,6-Diaminopurine — 6-Chloropurine — Purine

6-Mercaptopurine — 6-Thioguanine

FIG. 14.—Purine antagonists.

occasional improvement has been obtained in reticulosarcoma, but it is of no value in chronic lymphatic leukaemia, Hodgkin's disease, myelomatosis, and various disseminated cancers. It has the advantage over the folic acid antagonists of being effective in adults as well as in children, and the cytological variety of the disease has less influence on the chance of success. The general finding is that, in acute leukaemia, remission is procured in about one-third of the children and one-seventh of the adults treated (Bross, 1954). The drug is given by mouth, in doses equivalent to 2.5 mg. per kg. body weight ; toxic effects other than marrow aplasia are rare. Remission may not be established until after eight weeks of treatment. Its average duration is between four and five months, but in occasional instances it has exceeded a year.

A number of other purine antagonists have been tested clinically. *8-Azaguanine* proved toxic and ineffective (Armistead *et al.*, 1949). *6-Thioguanine* has a similar range of activity to 6-M.P., but offers no advantages over it (Murphy *et al.*, 1955). The same may be said of *6-chloropurine*, with the reservation that the frequency of remission may be somewhat higher in patients over the age of 40 years (Murphy *et al.*, 1955). *6-Methyl-mercaptopurine* will also induce remission in acute leukaemia, but no extensive clinical trials have been reported (Hall, 1956). *Purine* itself proved unduly toxic (Burchenal, 1956). Finally, *puromycin*, or *stylomycin*, an antibiotic derived from *Streptomyces albo-niger*, has been shown to be a purine antagonist and to be active against animal tumours (Oleson *et al.*, 1955). Clinical trials, however, have proved it ineffective in human disease and to cause renal damage (Wright *et al.*, 1955 ; Burchenal, 1956).

After an interval, acute leukaemia, initially responsive to 6-M.P., becomes refractory, and thereafter the disease is resistant to other purine antagonists. There is, however, no cross-resistance between these drugs and the folic acid antagonists.

Other Antimetabolites

No other antimetabolites have a firmly established place in therapeutics. Of the pyrimidine antagonists, *2-thiouracil* was used some years ago in the treatment of leukaemia, because it had been noted occasionally to cause agranulocytosis in patients with hyperthyroidism. It will sometimes depress the leucocyte count, but seldom leads to clinical improvement (Limarzi *et al.*, 1946). *Amicetin*, an antibiotic with anti-pyrimidine effect, has proved of little value clinically (Tan and Burchenal, 1956).

DL-*Ethionine*, an antagonist of the amino-acid methionine (White and Shimkin, 1954), and *selenium-cystine*, probably a cystine antagonist (Weisberger *et al.*, 1956), have both proved too toxic for use in man.

Azaserine (Fig. 15), an antibiotic derived from a *Streptomyces*, acts as a glutamine antagonist (Hartman *et al.*, 1955). It will induce transient remission in some children

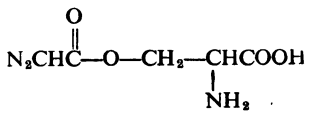


FIG. 15.—O-diazoacetyl-L-serine (azaserine).

with acute leukaemia (Ellison *et al.*, 1954), and there is a suggestion that, used concurrently with 6-M.P., it may increase the frequency of satisfactory response. This possible synergistic effect is under investigation at present.

CONCLUSION

This brief account of some of the agents employed to-day in the chemotherapy of malignant disease suggests that the effort expended by workers in this field has not been rewarded by commensurate achievement. To the clinician, impotent in the face of disseminated cancer, the melancholy catalogue makes disappointing hearing. For him the only yardstick is cure, and he must regard it as unrealistic to be gratified by partial success. Nevertheless, he must reflect that this field has been cultivated for less than twenty years ; that disease processes hitherto unrelentingly progressive may now be halted for a spell ; and that patients desperately ill may for a time be restored to health. During these few years the foundation of a logical approach to the problem has been laid and an efficient machinery for selection and testing of remedies devised. The fruits of these endeavours, if they do little to lighten our task at the bedside to-day, allow us to look with reasoned optimism to to-morrow.

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VIRUS OF ACUTE ENCEPHALOMYELITIS OF MAN AND MULTIPLE SCLEROSIS

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In the spring of 1957 our colleague Dr. R. S. Allison received a request to obtain for patients with multiple sclerosis a vaccine which has been studied in Russia and for which some success had been claimed (Choubladze and Gaidamovitch, 1956a). This vaccine consists of a formolized suspension of the brains of rats or mice which have been infected with the SV strain of a virus of acute encephalomyelitis of man—encéphalomyélite humaine aigue (E.H.A.) (Specification of the State Medical Testing Laboratory, Moscow*). It is manufactured and distributed by the Metchnikoff Institute in Kharkov, where it is called the "vaccine of Margoulis and Choubladze." Before this vaccine was tried in Northern Ireland it seemed desirable to know more about the virus from which it was made and the reasons for using it.

SV Strain of E.H.A. Virus

The E.H.A. virus was recovered in 1942 by Margoulis, Soloviev, and Choubladze in the course of research on the

*"Specification for the preparation and testing of vaccine for the treatment of patients suffering from acute disseminated encephalomyelitis, from disseminated sclerosis, or from a conditior brought about by an antigen for intracutaneous diagnostics.' State Medical Testing Laboratory, Moscow.