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NEW DEVELOPMENTS IN CARCINO-CHEMOTHERAPY*

BY

F. BERGEL, F.R.S.

Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, London

The clinical treatment of all forms of cancer, including leukaemias and reticuloses, rests increasingly on combined operations of surgery, radiotherapy, and chemotherapy (Table I).

TABLE I.—Treatment of Cancerous States

Surgery	Radiotherapy	Chemotherapy
Pathologist Surgeon	Pathologist Radiotherapist Biophysicist	Pathologist Biochemist Experimental pathologist Chemist Clinician

While in the past surgery or radiotherapy or both were the sole methods to alleviate or cure malignant diseases, chemotherapy as the youngest member of the triad, after its victories in the battles against bacterial, spirochaetal, protozoal, and, so far, a few viral infections, is coming slowly into its own also in the field of neoplastic and related states. It is true, however, that there the systemic use of drugs has not the same degree of success as against foreign invaders (their effects, particularly *vis-à-vis* leukaemias, are more of a palliative than of a curative character), but their combination with either radiotherapy (additive or synergistic) or surgery (perfusional (Abel, 1960) or post-operative) or both is promising (Fig. 1). Such limited favourable results may give the experimentalist a little more time to find better remedies and to bridge the gap between the causation and behaviour of animal and human cancers.

FIG. 1.—Relationship between chemotherapy, radiotherapy, and surgery.

Groups of Agents

Below is given a brief review of the main groups of chemotherapeutic agents at present in use. This is followed by a presentation of some work in progress—by necessity of an experimental character—aiming at less toxic and more selectively acting remedies and at a possible increase in the susceptibility of the neoplasms to be attacked.

TABLE II.—Anti-tumour and Anti-leukaemic Agents

- Biological Alkylating Agents
 - nitrogen mustards
 - methanesulphonates
 - ethylenimines, etc.
- Antagonists and antimetabolites:
 - Altered pyrimidines
 - purines
 - pteridines (folic acid)
 - vitamins (riboflavine, pyridoxine, nicotinamide, vitamin B₁₂)
 - amino-acids and peptides
- Products from micro-organisms and plants:
 - Polysaccharides, antibiotics, podophyllotoxin, colchicin, etc.
- Enzymes and co-enzymes:
 - Xanthine oxidase, ribonuclease, pyridoxal phosphate-metal complexes
- Hormones and antihormones
- Immuno-bodies

In Table II the first group comprises the *biological alkylating agents*, so called because their main action consists in altering essential cellular constituents, such as nucleic acids, proteins, and enzymes, by alkylation and cross-linking, processes which lead to cell death. Among these agents are the *nitrogen mustards* (Fig. 2)

FIG. 2.—Biological alkylating agents.

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with their warhead $N(CH_2CH_2Cl)_2$ —examples, HN2, originally designed as war gas and now used during regional perfusion, its oxygen derivative nitromine, an aromatic acid, chlorambucil, which is clinically used in lymphocytic diseases; the *methane sulphonates*, such as busulphan ("myleran"), the standard drug for chronic myelogenous leukaemia; the *ethylenimines*, tepa, thiotepa, used clinically also in perfusions, and Bayer E39; and, finally, the *epoxides*, which are not very frequently applied.

The second group in Table II comprises the anti-metabolites or metabolic antagonists, which as fraudulent substrates, products, cofactors, etc., are supposed to interfere with normal cell processes. Examples are (Fig. 3) 6-mercaptopurine and 5-fluoro-uracil, and 6-aza-uracil derivatives as antipurines and pyrimidines and aminopterin and methotrexate ("amethopterin") as antifolic-acid agents.

The third group represents products of micro-organisms, such as antibiotics, actinomycin, mitomycin, or of higher plants. This is followed by the as yet purely experimental group of enzymes and coenzymes, to which we shall return later.

Finally, although belonging to the disciplines of endocrinology and immunology, there are the hormones, such

as oestrogens—that is, stilboestrol (against cancer of the prostate) or androgens (against cancer of the breast)—and possibly antisera or vaccines.

Improvements of Cytotoxic Agents Sought and Achieved

After this "Cook's tour de force" an attempt should be made to answer the question: What is going on now in some laboratories all over the world to meet the challenge of the physician who demands improved drugs for systemic treatment and the more recent challenge of the surgeon who requires agents of reliability and safety for his regional perfusions? Time does not allow me to do justice to all contributions, but a selection of present-day investigations is offered.

Phenylalanine Derivatives

Starting with the postulates that the carrier moieties of biological alkylating agents are just as important as the "warheads" and that molecular structures related

FIG. 4.—Phenylalanine derivatives.

FIG. 3.—Antipyrimidines, antipurines, and antifolic acids.

to naturally occurring compounds might have advantages, some laboratories chose amino-acids, others sugar-like units, and, again, others pyrimidines as subjects of their studies which are being pursued in Britain, the United States, Japan, Hungary, and Russia. At the Chester Beatty Research Institute the chemists became interested, among others, in the phenylalanine (Bergel, 1958; Stock, 1958) series (Fig. 4). The *p*-mustard compound is now known as melphalan or PAM, in its racemic form as merphalan, or in Russia as sarcosylsin (Larionov *et al.*, 1955). That melphalan is effective on human melanoma cells in form of tissue culture has been demonstrated by Ambrose *et al.* (1961), and is shown in Fig. 5 in comparison with chlorambucil.

Apart from placing the mustard warhead in different positions of the aromatic ring (*o*- and *m*-analogues studied in London by Connors *et al.*, 1960, and in California by Gram *et al.*, 1959; Osdene *et al.*, 1959), which, like the steric arrangements of the carrier moiety (L, D, DL) (Bergel and Stock, 1953), has a measurable effect on the anti-tumour activities (Walker carcinoma 256, S91 Cloutman mouse melanoma), the workers at the C.B.R.I. increased the size of the molecule by combining the original melphalan with a great variety of amino-acids (see Fig. 4). To mention only two examples, so-called N-acetylphenylalanyl-melphalan ester (CB 3224) and L-leucyl-melphalan ester (CB 3262) (Bergel and Stock, 1960) gave, so far only in animals, promising results, the former possessing an improved therapeutic index—that is, less toxic in proportion to its anti-Walker effect, the latter apparently gaining carcinostatic activities against a greater range of experimental tumours (S180 in the mouse, and sarcoma induced with benzpyrene in the rat) which had so far proved more resistant to other related compounds.

FIG. 5.—Effect of melphalan and chlorambucil on human melanoma cultures. After one day's and after two days' treatment. (By courtesy of Dr. E. J. Ambrose.)

Dimethanesulphonic Acid Esters of Sugar Alcohols

Another investigation under the direction of Timmis (Haddow *et al.*, 1958), who is the discoverer of the well-known antileukaemic drug myleran, led to the preparation of the methane sulphonate-diester of the sugar alcohol mannitol (Fig. 6). While its clinical testing is proceeding in America, the experimental data disclose a wide spectrum of activity with low toxicity and haemotoxicity.

"Latent" Drugs

This brings the review to another recent approach. So that the selectivity of agents for tumour tissues as against normal dividing tissue (bone-marrow, intestinal mucosa) would increase, a number of workers have

taken an interest in so-called "latent" drugs—that is to say, drugs which are inactive but could become activated under certain *in vivo* conditions. While Danielli and his school (Danielli, 1961) have pursued this matter on a speculative basis, Ross and Warwick (1956) were the first to produce a deactivated azo-derivative of an aromatic mustard, CB 1414 (Fig. 7), which has aroused some interest in Canada and the U.S.A.

FIG. 6.—Dimethanesulphonic acid esters of sugar alcohols. (From Timmis and Brown, 1960.)

FIG. 7.—Reductive activation of chloroethylamino-azobenzenes. (By courtesy of Dr. W. C. J. Ross.)

Another grouping which keeps the warhead

in check, unless it is removed, is next to it either P=O as in phosphoric acid, or C=O in amides. A representative of the former is the drug cyclophosphamide ("endoxan," "cytoxan") (Fig. 8), synthesized by the Asta

FIG. 8.—Formula of cyclophosphamide.

Werke in Germany (Arnold *et al.*, 1958), the idea based on the original work of Friedman and Seligman (1954) in the U.S.A., and of the latter a carbamyl serine derivative (mercasin) (Fig. 9), prepared by Wade (Bergel

FIG. 9.—Formula of carbamyl serine mustard, mercasin.

and Wade, 1959). Both drugs depend on a breakdown in the body, maybe induced by enzymic action (phosphoramidase and a "carbamase" respectively) to the active di-2-chloroethylamine (nor-HN2). While cyclophosphamide (Cancer Chemotherapy Reports, 1960) is in clinical use, mercasin, which additionally might be able to interfere with amino-acid metabolism, still awaits testing in man.

Antibiotics and Plant Products

Recent advances in the field of antibiotics and higher-plant products as anti-tumour drugs include the findings of Maddock *et al.* (1960) that Waksman's actinomycin D, a complex peptide, potentiated x-ray irradiation in mice and men, and that when applied simultaneously in cases of cancer in children (Farber *et al.*, 1960) (Wilms's tumour, etc.) led in a number of them to clinical cures. Otherwise there exists a constant stream of mould brews emanating mainly from industrial laboratories which are the subject of systematic large-scale tests by the American C.C.N.S.C., and from which from time to time an antibiotic with anti-tumour or antileukaemic effects is isolated. An older example is the discovery of azaserine and DON (Fig. 10) (Reilly,

FIG. 10.—Formulae of glutamine (I), azaserine (II), and DON (III).

1958). The Japanese are also very active in this hit-and-miss venture, having isolated, among others, mitomycin (Cancer Chemotherapy Reports, 1959), which, while highly toxic, has a wide tumour spectrum.

Among higher-plant products colchicine from the autumn crocus, and, more recently, vincalkeboblantine (Noble *et al.*, 1958a, 1958b) from periwinkle have found entry into clinical medicine.

Metabolic Antagonists

It has been mentioned before that metabolic antagonists form a large part of the as yet slender armamentarium of the physician battling against leukaemias and neoplastic diseases. Work with respect to this group is proceeding at a considerable pace, perhaps not paralleled by a similar rate of practical achievements. There are so many building units and enzymic events in the cell with which one could interfere, the problem always being that such interference should preferentially

involve the tumour cell. The difficult synthesis of 2'-deoxy-5-fluorouridine has put at the clinician's disposal a less toxic substance than the simple pyrimidine itself. 6-MP, still one of the mainstays in the treatment of acute leukaemias, has been transformed by the chemists into derivatives with less pronounced side-effects. Almost weekly new representatives of purine, pyrimidine, pyridine, and folic acid analogues or their enzyme inhibitors are produced, but so far only small advances are being recorded concerning their capability of hitting the abnormal cell harder than its normal counterpart. And the acute forms of leukaemia, although held in check for short periods, still have to find their match.

Speculative Developments

A point has been reached where the further question has to be asked: Will a continuation of the extensive work as sketched out above in a very brief manner lead to drugs or remedies with significantly greater efficacy? Or will the answer, if there is soon an answer, come from a different direction, emanating from a more physiological or biochemical approach? There is no time here to discuss the possible developments in the field of hormones, of immunological reactions, and of virology. Suffice it to say that the hormones, whether connected with sex organs or belonging to the corticosteroids, had, and have, some successes. The studies of antigen-antibody-complement properties of tumours are in their infancy (Zilber, 1961). Workers are battling with many difficulties, not the least those related to the distinction between the antigens of normal tissue, and additional, or missing ones, of neoplasms. And what about the viruses either as a causation or as a treatment of a few malignant states? From a purely clinical point of view they are at present just as elusive (Moore, 1961).

There remains one more speculation. If the development and establishment of malignancy were at least in part due to the loss of certain essential cellular constituents or to a faulty biosynthesis of one or another of them, would it be possible to initiate a replacement or substitution therapy which might achieve a degree of success at least similar to that with vitamins and amino-acids or hormones in cases of nutritional or endocrine deficiency diseases? A rational approach could only start with a detailed knowledge of all the biochemical properties and behaviour of the aberrant cell. Unfortunately we are still a long way from such a goal. More and more cases have been reported where

FIG. 11.—Spontaneous mammary carcinoma in C+ mouse.

quantitative differences in cellular constituents between cancer cells and comparable normal ones have been established, but no certainty exists whether these differences are of a primary or a secondary nature and whether they form a steady characteristic of histologically similar tumours. However, let us consider one example studied experimentally.

1. The enzyme activity per mammary cell increases during normal growth of breast.
2. The enzyme activity per mammary cell is lower in the cancer-susceptible than in the cancer-resistant mouse.
3. The enzyme activity per tumour cell is very low in comparison to the breast cell. Whether this fall in enzyme content per cell is associated with a fall in enzyme concentration within the cell is under study.

(a) μ moles $[+10^{-5}]$ xanthine oxidized / 1×10^{-8} nuclei/min.
(b) Nuclei/mg. wet tissue.

FIG. 12.—Xanthine oxidase during carcinogenesis in mouse breast. (From Bergel *et al.*, 1957, p. 256.)

A number of years ago Haddow (Bergel *et al.*, 1957) put forward the working hypothesis that the enzyme xanthine oxidase, because it controlled the purine pool from which nucleic acids would derive and consequently regulate cell growth and division, might arrest cancer growth when injected into tumour-bearing animals. One of the few spontaneous tumours in the rodent, available to the experimentalist, is the mammary carcinoma in the so-called C3H and C+ mouse (Fig. 11). Lewin *et al.* (1957), while working at the C.B.R.I., established that there was indeed a deficiency of the enzyme in the tumour as compared with precancerous and completely normal breast (Fig. 12).

When de Lamirande (Haddow *et al.*, 1958) repeated Haddow's preliminary trials by injecting highly purified xanthine oxidase from cow's milk as prepared by Bergel and others (Haddow *et al.*, 1958), an arrest in the growth rate of the tumours occurred (Fig. 13).

Is it possible to find a comparable biochemical for injection into the human patient suffering from one or the other form of cancer; would it be possible to overcome immunological complications if the material had antigenic properties; would it be active against a tumour in man in the

FIG. 13.—Effects of xanthine oxidase on spontaneous mouse mammary carcinoma. ○, 18 treated mice. ●, 18 control mice given saline.

way in which insulin of bovine origin, while not curing diabetes, controls the pathological effects of the disease? We do not know yet.

Summary

A considerable number of drugs for systemic and perfusion chemotherapy are at the disposal of the clinician. They are at present not ideal remedies, and thus have to remain in the hand of the specialist. However, improvements in toxicity and selectivity may follow the lines presented above. The practical use of immunotherapy and replacement therapy in clinical medicine depends very much on future progress, and, so it is believed, to a large extent on a concerted effort in many laboratories to create a series of tests which would assist in a biochemical characterization of human cancers. This, together with the old-established morphological description as given by the pathologist, should enable the clinician in collaboration with the practitioner to arrive at the right choice of treatment—surgery, radiotherapy, or chemotherapy, or any of their combinations—to remove the deadly sting from these up to now far too often killing diseases.

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