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CHEMOTHERAPY IN THE TREATMENT OF MALIGNANT DISEASE*

BY

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The title of this discussion is a little unfortunate in that it would imply that there really is a chemotherapy of cancer. A less misleading title would have been something like "Empirical Attempts at Chemotherapy of Cancer." The term in its classical sense presupposes that one understands exactly the nature of the disease one is attempting to cure. The first example was the use of arsenicals by Ehrlich in the treatment of syphilis, terminating in the triumphant production of "salvarsan" in 1911. Here the mode of infection was known, the nature of the infective agent—the spirochaete—had been demonstrated, and the general pathology of the disease was completely understood. The same applies to the other successes in this field, such as the chemotherapeutic attack on malaria, generalized infections, and so forth.

It is not an overstatement to say that we have no knowledge whatsoever of the causation of the great majority of human cancers, nor have we really any proper understanding of the malignant process which enables cells to multiply and divide and so destroy the host. In certain types of cancer we do know the predisposing factor—for example, in the tar tumours or the tumours in the bladder caused by contact with certain chemical substances in the dyestuff industry. However, we do not know why these agents turn innocent cells into malignant cells, nor what the difference is between the malignant cell and the non-malignant, other than its power of unlimited growth. We cannot stop it once it has been started, except by some ablative form of treatment such as surgery or irradiation. We must therefore admit that anyone attempting chemotherapy is truly working in the dark, and that he must of necessity be an empiricist.

I shall not attempt to give anything like a complete list of the various substances and preparations that have been used in the chemotherapeutic attack on cancer. There is no satisfactory approach to the cancer problem in this way, and most of the preparations that have been used have had a very short vogue and then, with few exceptions, have been abandoned. It is with what can be saved out of the wreck, so to speak, that we are concerned.

Two Groups of Effective Substances

The substances which have an effect on malignant disease may be classified under two headings: (1) those which do not affect the cancer itself but which affect the host background in such a way that growth of the malignant tumour is either not favoured or is suppressed and held in abeyance; (2) those which exhibit a directly poisonous action

on the cancer cells, acting preferentially on them as compared with the host tissues.

The former substances are well known and are not of fresh interest. One of the most striking examples is the use of stilboestrol, or synthetic oestrogens, in the treatment of carcinoma of the prostate. Stilboestrol is highly active when given by mouth and can replace the naturally occurring substances in every way. When Huggins and Hodges (1941) wished to damp off the activity of the anterior lobe of the pituitary in cases of carcinoma of the prostate they utilized this powerful orally active oestrogen. By cutting off the supply of gonadotrophic hormone to the testes the secretion of androgens is abolished, and carcinoma of the prostate is unable to flourish in a background that does not contain androgens. Stilboestrol therefore produces its good effect, we believe, not by a direct action on the tumour but by altering the background or environment of the tumour so that it cannot flourish. It would appear also that certain carcinogenic hydrocarbons, when given to tumour-bearing animals, alter the balance of some nutritional factor, and it would seem doubtful whether the observed inhibition is due to a direct action on the tumour.

Turning to the second group, we encounter those substances which possess what is known as cytotoxic action. It is well known that a number of chemical substances are capable of preventing mitosis and thereby hindering cell division. An example of these is colchicine. This substance, however, not only inhibits the division of malignant cells but also affects normal ones, and therefore cannot be used as a chemotherapeutic agent. The great toxicity to normal tissues of all the known cytotoxic agents makes their use so hazardous that it is doubtful whether they should be employed.

Two of the most important of the substances used are urethane and nitrogen mustards. The fact that urethane could be employed as a cytotoxic substance was first discovered by Haddow and Sexton (1946). Nitrogen mustard is an analogue of mustard gas, and the one most commonly employed is methyl-*bis*- β -chloroethylamine. It is known that mustard gas itself has a powerful action on the nuclear mechanism, and that it is able to produce mutation by affecting the chromosomes of the *Drosophila* (Auerbach, Ansari, and Robson, 1942, 1943). Careful clinical experimentation has shown that nitrogen mustard seems to have a slightly more toxic action on certain malignant cells than on normal cells.

Folic Acid and Allied Compounds

The greater part of my space will be devoted to the newest comers in this field of would-be chemotherapeutic agents—namely, the folic acid and anti-folic-acid group.

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It is very difficult for anyone who has not visited the United States to realize the tremendous concentration of both laboratory and clinical workers on this group of substances. As the literature on the subject is diffuse, a brief summary of the general position might help readers to get a better grasp of this admittedly complex subject. To indicate how the whole subject of folic acid and allied compounds and their application to the treatment of cancer actually arose it is necessary to review a series of simultaneous researches in relation to malignant disease.

From 1939 onwards appeared a series of papers by Lewisohn *et al.* (1940, 1941a, 1941b, 1942). This work described a search for substances capable of causing regression of spontaneous mammary tumours in female mice of Rockland Farm strain. It was claimed that regression occurred when these animals were treated with extracts from such varied materials as spleen and certain cereals. Attempts to isolate the substance common to all these various extracts yielded only inositol, and it was claimed that this was apparently responsible for the inhibition. These experiments were continued in 1944 (Lewisohn *et al.*, 1944, 1945) and were the subject of a certain amount of controversy. We must now turn to other researches.

A whole series of workers were studying the growth of bacteria on purified synthetic media. Growth of a number of organisms could be stimulated by the addition of extracts of various substances such as spinach, spleen, and liver. It is neither desirable nor possible to review this work here, but it can be stated that as a result of chemical investigations it became obvious that one factor was responsible for these effects, and that this factor also appeared to be identical with the one used by Lewisohn and his collaborators.

A further series of researches, started in 1935 by Day *et al.* (1938), showed that a certain type of anaemia induced by deficient diet in monkeys could be cured by the addition of extracts of liver, spleen, and green leaves. They called this substance "vitamin M." Again chemical investigation of this suggested that it was possibly the same substance as that mentioned above. A precisely similar story was disclosed in relation to the dietary factors responsible for the growth of chickens and the proper development of their feathers. The substance was identified as pteroylglutamic acid, and the name "folic acid" was adopted for it. The formula of folic acid is:

It has since been synthesized by SubbaRow and his collaborators (Angier *et al.*, 1945, 1946; Waller *et al.*, 1946), and possesses the following characteristics: it crystallizes as yellow pear-shaped platelets; it is sparingly soluble in water (the sodium salt is much more soluble); its physiological properties show that it has a very low degree of toxicity and that it is an essential growth factor for a number of bacteria, animals, and birds. Clinically, its most important action is in Addison's anaemia, in which it is able to cause a haematological remission in the same manner as the liver substance. It will be remembered that it does not protect against the neurological complications, and is therefore different from the liver substance. This we now know

to be a crystalline cobalt-containing substance, known as vitamin B₁₂. This was isolated by Lester-Smith (1948) and also by workers in the Merck laboratory in the United States (Rickes *et al.*, 1948).

A further complication is that folic acid may exist free in the tissues or as a peptide. Two such peptides have been synthesized. Whilst folic acid is pteroylglutamic acid, there is pteroyldiglutamic acid, which has been called diapterin, and pteroyltriglutamic acid, which is known as teropterin. In the body these substances are digested, releasing folic acid. Therefore their administration to the human subject has the same effect as the administration of folic acid.

I now return to the researches of Lewisohn and his colleagues. They described the cure of breast carcinoma in three different strains of mice and showed that the active part of their extract was identical with the pteroyltriglutamic acid, or teropterin, referred to above. At a later date SubbaRow and his collaborators (Boothe *et al.*, 1948) synthesized both diapterin and teropterin, and these substances became available in relatively large quantities for clinical research. It is not necessary to review in detail the investigation of the anticancerous activity of these substances, but it can be stated that to-day it is admitted that they are without value in the treatment of both animal and human cancer. The good results obtained by earlier workers in human malignant disease were undoubtedly due to psychological effects, and when carefully controlled experiments were made with blank controls no specific anticancerous effect could be attributed to either teropterin or diapterin. This chapter can therefore be regarded as finally closed, and it must be admitted that neither folic acid nor its peptides are of any value in the treatment of malignant disease.

Modified Folic Acids

I must now turn to further chemical research. The synthesis of teropterin renders possible the preparation of a series of modified folic acids. The numbering of the atoms in the ring systems is shown in the folic acid formula above, and various substitutions can be made. Thus instead of glutamic acid it is possible to make a compound in which aspartic acid replaces the glutamic acid. The compound, which is known as an-fol-A or R, has the following formula:

Similarly, it is possible to take the pteric acid and prepare methylpterioic acid, which is known as met-fol-B and whose formula is as follows:

Another compound is obtained by substituting an amino group in the 4-position, giving 4-aminopteroylglutamic acid, or "aminopterin." Similarly, in addition to substitution of an amino group at the 4-position, a methyl group

can be attached, as in methylpterotic acid, giving 4-amino-methylpteroylglutamic acid, or A-methopterin. Finally, the aspartic acid compound can be substituted at the 4-position by an amino group, giving 4-aminopteroyl-aspartic acid, or amino-an-fol.

When these substances were tested out physiologically it was found that they possessed very powerful anti-folic-acid activity. The modern conception is that folic acid is essential for the growth of normal cells. This is proved by tissue-culture experiments and by experimental feeding in animals, and of course it is well known to be essential for the growth of bacteria. It will be remembered that Fildes and Woods (1940) showed that sulphonamides act by replacing the normal constituent of the bacterial nutritional requirements—namely, *p*-aminobenzoic acid. It was felt that a similar type of experiment could be done with the anti-folic-acid substances, and that just as one could deceive the bacteria, so to speak, by feeding them with sulphonamides, which could be inhibited by *p*-aminobenzoic acid, so it would be possible to palm off one of these five anti-folic-acid substances on the cells, these substances becoming fixed in the cells so that they ultimately died. The folic acid antagonists act *in vitro* in tissue cultures and will also inhibit the action of folic acid on the growth of bacteria. In mammals they produce a powerful inhibition of the bone marrow, resulting in severe anaemia—effects similar to those seen in folic acid deficiency.

Since these substances interfere with the multiplication of cells, probably acting on the nuclear mechanism, their trial in malignant disease was obviously indicated. They have been investigated extensively for their action on spontaneous and transplanted tumours, and have been found to be effective against a number of them. They have also been used in the treatment of human malignant disease, and particularly in the leukaemias.

Conclusion

It must be pointed out that these bodies are extremely toxic and their use in the human subject is fraught with danger, as the toxic dose is so near the effective therapeutic dose. As Rhoads (1948—personal communication) has so rightly pointed out, it does not really matter from the experimental point of view how close the toxic dose is to the therapeutic dose, provided it can definitely be shown that these substances do exert a specific toxic action on malignant cells. The question of the moment is whether in this series we have the starting-point of a new set of compounds for the treatment of malignant disease; in other words, do these anti-folic-acid analogues occupy the same position in relation to the ultimate chemotherapeutic control of malignant disease as “atoxyl” did to salvarsan?

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Treatment of malignant disease by medicinal means has been tried for many years, and the very multiplicity of the suggested remedies and their short-lived popularity serve only to indicate that none, so far, has proved of value. It must be emphasized that there is no specific remedy which destroys a malignant growth except surgical ablation or radiotherapeutic destruction of the tumour. Nevertheless, advances in biochemistry and biophysics have resulted in the production and use of chemotherapeutic substances which have an effect either on the tumour or on the host, and which so alter the natural course of the disease as to effect remissions, although these are only temporary. The importance of these chemotherapeutic trials lies in their scientific implication; they are pointers or indicators for further lines of research. They are not curative agents, and only a few can be said to be even of palliative value in the control of the disease. It is, however, important from time to time to review present-day knowledge.

The remedies under clinical trial to-day fall into two main groups: (1) those with a cytotoxic effect on the neoplasm, and (2) hormones which alter the “internal carcinogenic environment” of the patient. This expression we owe to Cramer and Horning (1939).

The most interesting substances of the group of cytotoxic poisons are the nitrogen mustards, urethane, and the anti-folic-acid compounds.

Nitrogen Mustard

Nitrogen mustard was first introduced in 1942, and has been used clinically as the “HN2” compound (methyl *bis*-(β -chloroethyl) amine); the “HN3” compound (*tris*-(β -N-chloroethyl) amine) being more toxic and producing widespread thrombosis. The dose generally used is 0.1 mg. per kg. of body weight, given intravenously up to a total of 0.6 or 0.8 mg. per kg. of body weight. It has been tried in many types of tumours—bone sarcoma, melanotic carcinoma, the reticulosos, Hodgkin's disease and lymphosarcoma, carcinoma of the lung, and testicular neoplasms. In some cases visible regression of large tumours, especially masses of enlarged lymph nodes, has been obtained, with a corresponding improvement in the general state of the patient. So far as I am aware there is, however, no recorded case of an apparent regression for prolonged periods. Nitrogen mustard is now used almost exclusively in the terminal stages of lymphadenoma and in cases of cancer of the lung untreatable by surgical or radiotherapeutic methods. It is of palliative value and may restore health for short periods of time—up to nine months in my own experience, but generally for three months only.

The fact that cures have not been obtained should not be a deterrent—on the contrary, it should be an incentive—to the research chemist and biochemist in their search for other mustard compounds more effective and possibly less toxic. Regression of neoplasms, although temporary, is nevertheless very dramatic. Thus in Hodgkin's disease, at a stage resistant to radiation, with fever of either the Pel-Ebstein intermittent type or even the sustained pyrexia type

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