Rational Cancer Chemotherapy

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THE DEVELOPMENT OF CHEMICAL MEANS for restraint of neoplastic disease has passed through two well-defined phases. It is now entering a third. The first is marked by the discovery of agents, or procedures, which cause atrophy of specific tissues and, by virtue of this property, induce restraint of neoplasms derived from them. The hormonal treatment of cancer of the prostate, breast, and of leukemia is, of course, of this first type, as in the use of the radioactive isotopes of phosphorus and iodine.

The second phase is the one of the use of compounds which probably are injurious to cells in direct ratio to their rates of growth. Since many, but not all, neoplastic cells are actively reproducing, they are particularly susceptible. The compounds related to the nitrogen mustards and those acting as antimetabolites to folic acid presumably are in this group.

The third phase has just been entered with the study of substances which injure selectively by virtue of biochemical specificities of the target cells, characteristics which distinguish the neoplastic from their normal analogues growing at the same rate. This selective effect may well be truly specific cancer chemotherapy, now just in its earliest inception, but bright with promise for the future. The compound recently described, 6-mercaptopurine (*1–4*), represents perhaps a first step in the attainment of compounds with selective anti-neoplastic action in man. This possibility is particularly apparent when the results of use of the compound are considered together with the investigative work which led to its synthesis, and with an understanding that related compounds, acting even more selectively by similar principles, are already coming to the testing laboratory.

The compound, 6-mercaptopurine, is the product of a program instituted in 1946 in the laboratories of the Sloan-Kettering Institute. Subsequently, affiliations for complementary work were developed in the Burroughs Wellcome and Company (U.S.A.) Incorporated and the Southern Research Institute. The program was established under five divisions, with functions as follows:

1. The discovery, development, and use of precise methods of assay for measuring the ability of compounds to restrain selectively the growth of mutant as compared to normal cells—viral, bacterial, protozoal, and mammalian. The mutants include, of course, neoplastic cells of animal and human origin.

2. The synthesis, collection, coding, testing, and compilation of results of trial of candidate compounds for cancer chemotherapy.

3. The development of methods for assessing exactly the ability of substances tested to restrain cancer in man.

4. The organization of an extensive program for the application of these methods to patients of suitable types and in adequate numbers, with the training of qualified personnel to real competence in this exceedingly difficult field. It is difficult because it requires the study of desperately ill individuals, a thorough knowledge of the natural history of the various types of cancer, and a complete understanding of the pitfalls inherent in a therapeutic program dealing with a disease often slow in its progress and prone to unpredictable modifications of its course.

5. An orderly program for the comparative analysis of the patterns of nucleic acid anabolism in normal and mutant cellular forms, including the neoplastic in mammals. This work has been notably productive in its yield of new compounds and unique information.

Chemotherapy of bacterial disease came about almost entirely as the result of empiric study. Knowledge of the mechanisms of the chemotherapeutic effect was secondary—the consequence of the availability of selectively acting agents, rather than their source. The empiric approach is at best hazardous, ponderous, and cumbersome. It is one which involves many gambles at high odds. As a result, it is never popular, even though medical history is studded with notable examples of its success. Its first use, in bacteriology, was feasible because of the relative simplicity of the problem and the ease of the manipulations involved in its solution. The number of tests with bacteria that can be made in a given time is limited only by the funds available and the energy of the investigator. The organism whose destruction is sought is capable of artificial cultivation on any scale.

The situation in cancer chemotherapy is quite different, however. Here, though the target, the cancer cell, is well enough defined, it exists in many different forms, each obviously with its own biological characteristics. Furthermore, human cancer has been, until recently, impossible to cultivate outside the body.

Despite all these difficulties, many apparently insurmountable, an empiric attack on cancer was undertaken in our laboratory under Stock (*5*), employing a variety of transplantable animal neoplasms and transferring the results of their study to man as rapidly as possible. This program slowly developed information

which now permits the limited extrapolation of the results of animal tests to palliative cancer therapy in human beings.

To make the program properly inclusive and so as effective as possible, the empiric approach was amplified by the development and application of analytical methods for the comparative study of nucleic acid synthesis in normal and mutant forms. The results have accelerated the empiric work greatly and yielded new principles which appear to provide a rational approach to cancer control.

When the study of nucleic acid metabolism was instituted in 1946 by Brown, little information existed on the anabolism of nucleic acid in any cells, particularly the mammalian. The single experiment reported, that of Plentl and Schoenheimer (*6*), employing N^{15}-marked guanine, had failed to reveal any uptake of this substance by the nucleic acid of the rat. As a consequence, it was generally assumed that such dynamic equilibrium as existed in the case of nucleic acid depended upon purine and pyrimidine synthesis entirely from small, ubiquitous precursors, such as formate and glycine, widely distributed throughout the body. It was further assumed, since the amounts of desoxyribose nucleic acid (DNA) per cell, irrespective of type, were the same, that they were also identical, both in composition and in anabolism, in different cells.

The development of the one-gene, one-enzyme hypothesis, however, by Beadle (*7*) and his associates, gave rise to some consideration of the possibility that cells of different characteristics, and so certainly having different genes, might have DNA of different composition in those genes. Because of the minute size of the gene in comparison to the whole chromosome, the possibility of employing these conceivable differences to develop a chemotherapeutic approach (the selective destruction of certain cells while sparing others) was not at first considered seriously. It remained for the observations of Avery and his co-workers (*8*) to provide unequivocal evidence of the specificity of DNA. This, in turn, established a firm foundation for the development of selectively toxic compounds.

The original demonstration was the classic one, that a pure DNA prepared from Type III virulent, encapsulated pneumococci would transform the offspring of Type II avirulent, unencapsulated pneumococci into the encapsulated forms from which the DNA had been derived. Furthermore, this transformation was permanent, an hereditary characteristic capable of being transferred from parent to offspring indefinitely. Since this original demonstration, the same principle has been shown to obtain for a variety of bacteria and probably for at least one pair of viruses. Clearly, in these instances the DNA capable of transforming is different from that in the organism which is transformed. This difference, it was thought, should be susceptible to chemical definition, and might be reflected in measurable anabolic dissimilarities between the two.

Clearly, two means for defining the heterogeneity of nucleic acid exist. One involves the demonstration of different compositions of DNA's of two sources, preferably those capable of exerting different effects, such as two transforming factors. The other is the proof of unequal rates of incorporation of the same precursor into different DNA's and different DNA fractions. The methods for defining nucleic acid composition in terms of purine-pyrimidine ratios are still not entirely satisfactory. Such data as have been advanced in support of differences in such ratios between cells of different types are suggestive, but still equivocal. Perhaps the most conclusive are those of Markham and Smith (*9*) for different strains of tobacco mosaic virus. Similar data have recently been advanced for two strains of rickettsia (*10*).

The recently reported discovery of two types of DNA by Bendich (*11*) and variations between different tissues in their content of these two types lends further weight to the thesis of cell-specific DNA constitution and anabolism. Such specificity should be tantamount to susceptibility to selective destruction by antimetabolites. The heterogeneity of DNA, which is so essential to the chemotherapist if he is to have a rational program, seems now to have been established beyond reasonable doubt. Furthermore, new data supporting this conclusion are now coming forward from many different laboratories. Most impressive is the recent demonstration by Brown and Watson (*12*), based upon the original publications of Bendich (*11*) and Cavalieri (*13*). Clear proof is presented that a differential binding of nucleic acid bases exists, since different nucleic acids yield different patterns of dissolution by graded concentrations of salt.

The data of Bendich (*14*) on the differential deposition in DNA of the carbon from administered carbon-14 formate are particularly impressive. Not only is the distribution of the tracer different between the two DNA's of different organs, but the entire uptake pattern seems to be characteristic for the particular tissue involved.

The second type of work has been more productive. It is based on the original demonstration by Brown and his associates (*15*) of the recovery of parenterally administered marked adenine in both PNA and DNA of mammalian tissues. This is the point of departure for a substantial part of the many studies presently under way, notably by Mitchell and Skipper (*16*). It was quickly followed by proof from Brown that the mouse differs from the rat in that it will incorporate guanine to a greater extent (*17*). Then came the evidence that the adenine uptake is strikingly different for different types of cells of the same species (*18*). This point was amply supported by studies from many laboratories indicating that mutant strains of bacteria differ radically in their purine and pyrimidine requirements.

The analogy between original (wild) and mutant strains of bacteria, and normal and mutant mammalian cells with neoplastic properties, is obvious. The ability selectively to kill mutant bacteria by withholding from the medium a purine required by mutant and not required by the parent original forms is well established. Though few regarded this ability as the

analogue of a foundation for effective cancer chemotherapy, it became the basis of the work in our laboratories. It led to an extensive program for the synthesis and test of candidate chemotherapeutic compounds there, and also in a number of cooperating units, notably by Hitchings[1] of Burroughs Wellcome and Company (U.S.A.) Inc. and Skipper of the Southern Research Institute. Extensive series of compounds were prepared as modifications of known or presumed precursors of nucleic acid, and studied, with a variety of assay techniques, by Stock, Philips, and co-workers (*5, 19*). The publications of Biesele (*20–22*) should be consulted, since many of the most outstanding results were achieved by this investigator.

One compound, 2,6-diaminopurine, studied almost simultaneously in the Burroughs Wellcome and Sloan-Kettering Institute laboratories, proved to be selectively injurious in tissue culture for certain neoplastic cells of animals as compared with normal cells growing at the same rate (*22*). This was the first proof that such a selective effect, even *in vitro,* could be achieved employing cells of equivalent rates of growth. It is of especial interest that this compound is actually incorporated into normal nucleic acid purines. Not only did the compound exert preferential toxic effects *in vitro,* but it was shown by Burchenal (*23*) to be therapeutically active *in vivo* in prolonging the survival time of mice with certain strains of experimental leukemia.

It has actually been possible indeed to destroy selectively a viral-like, intra-cellular particle, the kappa particle of "killer" paramecia, without destruction of the protozool cell containing it (*24*).

Another product of this program, 8-azaguanine, was studied in several laboratories. In Kidder's hands (*25*) it was found to be active in restraining the growth of certain animal neoplasms, particularly the mouse mammary adenocarcinoma EO 771, although Sugiura has shown it to be inert against other neoplasms (*26*). The studies of Biesele demonstrate beyond question the extraordinary specificity of the effects which can be exerted upon cells of different types by analogues of nucleic acid precursors and by numerous related compounds. Furthermore, they prove that certain types of substitutions in these analogues confer upon the compounds a higher degree of selectivity than do others. This was not only a scientific accomplishment of moment, but it also represents the surmounting of a particularly difficult and frustrating psychological hurdle to cancer chemotherapy. This was the widespread conviction that the selective destruction of cancer as compared with normal cells was impossible, since both had a common origin. The obstacle is now removed by the weight of evidence, and, we hope, for all time. It appears that the way to more effective cancer chemotherapy is open, though it may prove to be a long and arduous one.

The fact of predominant nucleic acid synthesis *de novo* and the possibility that many different pathways exist have been advanced in deprecation of efforts to achieve cancer chemotherapy by block of nucleic acid synthesis. These do pose problems, but there is no reason to regard them as insurmountable. Goldthwait and co-workers (*27*) advanced unequivocal evidence that antimetabolites of folic acid block effectively *de novo* synthesis from formate at least in normal mammalian cells. The acquisition of resistance to and eventual dependence on 8-azaguanine by a transplantable neoplasm studied by Skipper (*28*) is clearly associated with a lessened dependence on *de novo* synthesis and 100 times greater uptake of the C^{14} marked antipurine.

The whole concept of specific cancer chemotherapy depends upon the existence of specific anabolic patterns for nucleic acid by particular cell types including the neoplastic. There is good evidence that these exist for normal cells, although they will unquestionably turn out to be much more complicated and so presumably much more varied and specific than present methods permit us to define. Different, and so useful, specificities will undoubtedly be found to exist for different types of neoplasms. This has also been advanced as an argument by those who oppose work in cancer chemotherapy. The most pessimistic pronouncement on this point is one recently made that 1000 different agents will be needed for 1000 different kinds of cancer. This is unlikely, since perhaps the majority of the cancer deaths are due to disease of a few general types. Data already existing on human therapy indicate that the responses by minor variants within these types tend to be similar, even though not identical.

The crucial question is whether a particular cancer does differ substantially from normal tissues in its requirements for building blocks of nucleic acid, precursors which may be modified to act as antimetabolites. Skipper (*29*), in a most significant contribution, seems now to have answered this question. The animal and human cancers studied for their uptake of C^{14} guanine differed consistently from normal tissue and from each other. Each seems to have its own consistent and specific requirements.

The most recent product of this program, 6-mercaptopurine, has now been in general use since January 23, 1953. On that date it was distributed, with information concerning its use in the treatment of acute leukemia, to several of the leading clinical groups in the country. The experience with it has been now reported and evaluated. The substance, though not curative, is useful indeed. Let us hope that future results with compounds developed on a similar basis, for the treatment of other neoplasms, are as encouraging. Many now ill to death hope for surcease, and many will in the future be grateful for similar hope.

Different rates of uptake of the same precursor by different types of cells clearly have been established. Analogues of these precursors have been synthesized. Certain of them have shown the ability selectively to injure neoplastic as compared with normal cells *in vitro* and *in vivo*. The program has now been extended into related areas by other laboratories, notably those of Woolley (*30*) and Gellhorn (*31*). It will be sur-

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prising indeed if these principles and these compounds with their congeners do not yield new means for cancer control in man within the foreseeable future.

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The Fine Structure of Cellulose Microfibrils

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THE microfibrils detected in native cellulose with the electron microscope (*1, 2*) can be further disintegrated by means of ultrasonics (*3*), hydrolysis (*4*), or oxidation (*5*). Whereas the microfibrils show diameters of 150–250 A, the resulting elementary fibrils (or micellar strands) grade down to 90–70 A. Vogel (*6*) has found that these elementary fibrils are flat filaments, sometimes only 30 A thick. These ribbons anastomose laterally with each other. Their lateral aggregation is visible when ultrathin sections of ramie fibers are disintegrated in a blender. The plane of the ribbon must correspond to the (101) plane of the cellulose crystal lattice, since Mukherjee and Woods (*7*) find by x-ray analysis that cellulose particles of ramie and cotton produced by H_2SO_4 hydrolysis sediment are parallel to that plane.

Based on these facts, the amicroscopic[1] structure of a microfibril can be described by Fig. 1. It represents the cross section of a thin microfibril which is composed of several aggregated elementary fibrils (micellar strands).

The elementary fibrils consist of a crystalline core that is flattened parallel to the (101) lattice plane. This shape is due to a faster growth of the (101) plane, which is more hydrophilic (*8*) than the more slowly growing ($10\bar{1}$) plane. Therefore, more energy is needed to remove the hydration water from the (101) plane when adding a new layer of chain molecules. The crystalline core of the microfibrils is embedded in a cortex of paracrystalline cellulose (*9*). The insufficient order of the chain molecules in this cortex may be caused by the escaping water released on the occasion of the polymerization of glucose and the crystallization of the resulting chain molecules.

The paracrystalline cellulose is responsible for the aggregation of the elementary fibrils to form *microfibrils*. The tendency toward aggregation in the (101) plane is greater than perpendicular to it. As a result,

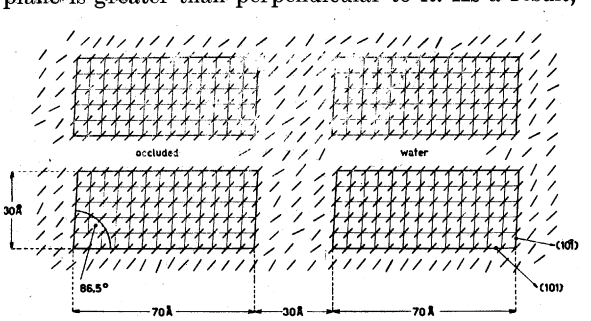

FIG. 1. Section across a microfibril of native cellulose composed of four elementary fibrils or micellar strands. A core of crystalline cellulose chains, seen in cross section, is embedded in paracrystalline cellulose. (101) and ($10\bar{1}$) planes of crystal lattice.

[1] *Amicroscopic*, particles less than 50 A, not visible in even the electron microscope.