Biological Approaches to Cancer Chemotherapy

Edited by

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FOREWORD

All those concerned with the design of drugs for cancer chemotherapy are intensely aware of the need for compounds with increased selectivity for tumours. This symposium was organized, under the auspices of the Cell Biology Panel of UNESCO, to consider this problem. The central issue is: how can we utilize information at present available, or potentially available, about the properties of living cells, in drug design? This symposium report is a preliminary and partial answer to this question. The full solution of the problem constitutes a theoretical problem of major importance, and of fascinating difficulty. The probability is that all of the necessary clues are available, and failure to solve the full problem results from the inadequacy of the intellectual effort so far put in.

The design of the symposium was drawn up by a preliminary meeting of many of the participants, held a year in advance of the full meeting. Both meetings were held at Louvain, in ideal circumstances provided by Professor J. Maisin and the University. The expenses of the meeting were defrayed mainly by UNESCO, with some assistance from WHO, and from divers other bodies in the form of travel grants to individual participants.

December 1960

CONTENTS

The following also attended the meetings and took part in the discussions: P. Rustin, L. A. Elson, S. J. Holt, J. Leiter, G. F. Marrian, G. Möller and A. B. Novikoff.

GENERAL CELL THEORY AND DRUG DESIGN

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SUMMARY

1. The selectivity of a drug for one cell lineage can be increased by increasing the number of variables upon which the action of the drug depends.

2. A general cell theory is outlined. Emphasis is placed upon the necessity for intracellular communication between the parts of a cell, as an essential part of cellular control mechanisms.

3. In designing chemotherapeutic procedure, we may either (*a*) design an agent to act upon the cell in its existing state, or (*b*) we may use cellular control mechanisms to modify cells and design drugs to act upon the cells so modified.

4. The design of agents under 3(*a*) is illustrated by some nitrogen mustards which can be activated by constitutive hydrolytic enzymes.

5. The design of compounds under 3(*b*) is illustrated by using induced enzymes as activating agents.

6. Other variables, e.g. permease and active transport processes, pinocytosis, lysosome properties, dissociation of gene duplication, may equally be used in drug design.

7. In the selection of a drug devised on these principles, information obtained by the examination of biopsy specimens from patients is essential.

THE MULTIPLE-VARIABLE PRINCIPLE IN DRUG SELECTIVITY

A large part of this paper is written on the general assumption that, with most "spontaneous" tumours, the differences between the tumour cells and the normal cells of the organism are small. And on the particular assumption that the difference with respect to any one variable is insufficient to obtain a therapeutic index adequate if all tumour cells are to be killed without intolerable damage to the organism as a whole. These assumptions are not necessarily true: they are made since, on the basis of information available now, they provide an adequate guide to action.*

Although the difference with respect to any one variable is assumed inadequate for effective drug action, if the small differences in respect of each of a number of different variables could be made additive, then

* Examples are not difficult to conceive in which an adequate difference could arise with respect to a single variable; e.g., if the causal agent is a virus, the immunological differences between host and virus could be adequate. If the cause is a Mendelian gene mutation then, as Haddow has indicated, replacement of the deleted gene product might be effective.

a clear therapeutic advantage could be established. This can be done by making the action of a drug dependent upon, not one, but several variables (Danielli, 1954; 1959a). The greater the number of variables involved, the greater the possible selectivity.

The possibility of using carcinolytic viruses may seem at first sight to present an exception to the principle that selectivity involves dependence upon a multiplicity of variables. But this is illusory. The action of a selective virus depends upon a complex of conditions, among which the following are included:

(*a*) The virus shall not be immediately removed by immune bodies.
(*b*) There shall be a correspondence between virus surface and cell surface leading to selective virus attachment.
(*c*) Penetration into cells must be procured either by specific enzyme action or by the induction of pinocytosis.
(*d*) The virus, on entering the cell, shall not be recognized as a foreign body and be destroyed by the lysosome mechanism.
(*e*) The host cell shall be competent, after infection, to synthesize all the viral macromolecules.
(*f*) The host cell shall provide an adequate site for assembly of virus from newly-synthesized macromolecules.
(*g*) Virus assembly shall lead to cell death.

When looked at in this way it is apparent that the procurement of a selective carcinolytic virus represents a biological approach to the problem of assembly of a lethal agent whose action depends upon several variables, i.e. a selective virus depends upon the multi-variable principle for its selectivity.

GENERAL CELL THEORY AND AVAILABLE INFORMATION FOR DRUG DESIGN

There is a large number of variables upon which drug action can be made dependent. So that the approach to this problem may be rational, i.e. not regarded as a "lucky dip", it is desirable to consider the cell as a whole, as far as is now possible. The following outlines many of the main points involved in a general cell theory (Danielli, 1959b).

Necessary Subdivisions of a General Cell Theory

A. *Intracellular control processes*

Control of nature of macromolecules present.
Control of amounts and proportions present.
Control of specificity of macromolecular interaction.
Control of organization into supermacromolecular units.
A mechanism for communication between the parts of a cell.

B. *Cell and environment interaction mechanisms*
Mechanisms for examining and reacting to environment.
Mechanisms for intercellular communication.

C. *Replication mechanisms*
Mechanisms for replication of macromolecules.
Mechanisms for replication of higher structures.
Mechanisms for cell division.
Mechanisms for gene distribution to daughter cells.

D. *Energy acquirement and conversion*
Mechanisms for acquiring free energy from environment.
Mechanisms for catalysis and control of catalysis.
Mechanisms for conversion of energy into diverse forms.
Mechanisms for restricting and increasing molecular fluxes.

E. *Mechanisms for genetic change*
Mechanisms for gene recombination.
Mechanisms for new gene acquirement.

In commenting upon this outline it must be emphasized that cells act as units, their activity being partly programmed by the information contained in their genetic systems, and partly determined by environmental factors. The essence of this unitary behaviour must lie in the cellular control systems outlined in Section A. The mechanisms involved are formally similar to those involved in electronic systems, provided it is borne in mind that intracellular communication is based upon chemical factors—intracellular hormones—and not upon electrical signals. The interior of a cell (and to some extent its environment) must be regarded as a conducting path carrying simultaneously thousands of different messages. In electronic systems most of the specificity of communication depends upon the existence of differentiated conducting pathways and upon the time intervals between signals. This is also true for communication between cells. But for communication within cells, where the conducting system is common to all communications, selectivity must depend upon the partitioning of the cytoplasm by innumerable membranes, the presence of selective permeases in these membranes, and in the correspondence between the chemical structure of the intracellular hormones, the structure of the permeases which facilitate their passage through certain membranes, and the structure of the ultimate acceptor. Thus, whereas with an electrical system much of the information content of a message is based upon a distribution of

signals *in the time dimension*, in a chemical system such as is involved by intracellular hormone action much of the information content is based upon the distribution of the signals *in one, two or three space dimensions*, e.g. with a steroid hormone the individual signals consist of simple chemical groupings firmly oriented in space by the steroid carbon skeleton.

We must envisage the intracellular hormone system as an essential part of the complex of feedback controls, threshold phenomena and cellular clocks which constitute the control mechanisms of cells.

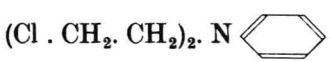
For our present purposes the feedback control systems are of essential importance. Much of the strictly biochemical aspects of such systems will be treated by Dr. Magasanik, but I wish to emphasize two of the physical properties of complex feedback systems, the significance of which does not seem to be generally appreciated in the context of cell biology, although it has been indicated by Goldacre on several occasions (1958a, b). Where feedback control is present, the system may oscillate between two extremes: this may constitute the physical basis for some cellular clocks. Alternatively, the system may have a number of alternative steady states. It seems probable that the different cell lineages which exist in an adult animal are in fact some of the alternative steady states in which a cell, with a given set of genes, may settle (Muggleton and Danielli, 1959). This being so, it is possible that tumour cells simply represent more of the alternative states.

I shall now consider how we may make the action of drugs dependent upon the action of a number of variables. First, utilization of some of the simpler properties of a cell will be considered, and subsequently use of some of the cellular control mechanism.

As a model system we have used the Walker rat sarcoma 256. The technique is essentially that of Haddow, except that treatment with a drug was delayed until the tumour had attained a weight of 6–10 grams (see Hebborn and Danielli, 1958). In the following tables PR indicates partial regression and CR indicates complete regression.

Drugs Designed to Attack the Cell As-It-Is

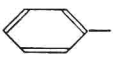
In a theoretical paper (1954) I showed how it was possible to make the action of a drug depend upon several cell variables. This has since been confirmed experimentally (Hebborn and Danielli, 1958; Danielli, 1959). Here we shall consider one simple example, data for which are summarized in Table I. The substance



. I

TABLE I. *Relationship between presence of activating enzyme and drug action.*

	% PR	% CR	Presence of activating enzyme
I R . NH_2	—	—	
II R . NH . CO . CH_3	70	20	+
III R . NH . CO . Ph	—	—	—
IV R . NH . CO . CH_2F	65	30	+
V R . NH . CO . CH(NH$_2$)(CH$_2$Ph)	60	40	+

R = $(Cl . CH_2CH_2)_2N$ 

is a mitotic poison. It is an extremely toxic substance both to Walker tumour cells and normal rat cells, the action being most pronounced upon cells approaching mitosis. It has little, if any, selective effect upon tumour cells. Its toxicity is due to formation of alkylating carbonium ions by ionisation of Cl^- from the β-chloroethyl groups. The rate at which this occurs can be modified by varying the other substituents in the benzene ring. For example, if the amino group of I is acetylated to . NH . CO . CH_3 as in II (Table I), the rate of ionisation is markedly depressed, and correspondingly the median lethal dose rises. But contact with an enzyme capable of splitting off the acetyl group will restore the original aggressiveness of the compound. Thus, whereas the parent compound I acts mainly with respect to the incidence of mitosis, the acetyl derivative depends for its action upon two variables—mitosis and presence of amidase. Thus II could be described as a mitotic poison with a proximity fuse set off by amidase. Walker tumour contains a significant proportion of enzyme able to liberate acetate from II, and it is interesting to see that II is strikingly more effective than I. Correspondingly, the relative inability of Walker tumour cells to hydrolyse the benzoyl derivative III is associated with absence of action upon the tumour. Compound IV, a fluoracetyl derivative, on hydrolysis releases the toxic fluoroacetate instead of acetate: there is an increase in antitumour action resulting from introduction of action upon a third variable—fluoroacetate inhibition of the Krebs cycle system. Compound V shows a marked increase in activity: it consists of I acylated by phenylalanine. It is activated by aminopeptidase; and it may well be concentrated in tumour cells by the transport mechanism for amino acids.

The use of other variables has been described in the papers mentioned

above. The theoretical prediction that dependence upon several variables would increase selectivity has been amply confirmed.

The Use of Control Mechanisms

The approach defined in the previous section was to make use of tumour cells as they are. An alternative approach is first to change the tumour cells so that they become less like normal cells, and design a drug which will act upon the cells so altered. This approach arose from considering the fact that tumours readily become resistant to a wide variety of drugs. In so doing they become less like normal cells, so that if the process conferring resistance could be utilized it should add to the therapeutic advantage.

Use of Adaptive Enzymes

Haddow and Sexton's (1946) finding that urethane initially inhibits the Walker tumour, but that this is soon followed by resistance, was taken as a starting point. In my original theoretical paper I suggested that the resistance was due to induction of an enzyme capable of splitting urethane, and that this same enzyme, once induced, could be used for drug activation. It has now been shown that a urethane such as Ph . NH . CO . OPr^i does induce formation of urethanase in the Walker tumour, and that subsequent treatment with the otherwise inefficient compound $(Cl . CH_2 . CH_2)_2N$  NH . CO . OPr^i is exceptionally effective in causing complete tumour regression. Thus the urethane acts as a potentiator for a urethane mustard.

TABLE II. *Data for potentiated mustards.*

	Potentiator	Mustard	% CR Unpotentiated	% CR Potentiated
A	Ph NH . CO . OPr^i	R . NH . CO . OPr^i	—	80
B	Ph . S . CO . OPr	R . S . CO . OPr^i	10	50
C	Ph . NH . CO . NH . CH_2 . COOH	R . NH . CO . NH . CH_2COOH		
D	Ph . S . CO . NH . CH_2 . COOH	R . S . CO . NH . CH_2 . COOH		
E	Et_2N  NH . CO . CH_3	R . NH . CO . CH_3	20	~ 50
F	Et_2N  NH . CO . CH_3	R . NH . CO . CH_2F	30	~ 50

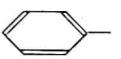
R = $(Cl . CH_2CH_2)_2N$  —

Table II summarizes the results obtained with six pairs of substances. In each case the potentiator has little, if any, capacity for causing tumour regression. The potentiator must be regarded as a means of preparing a tumour cell for decisive action by an induced-enzyme activated drug.

There are innumerable other possibilities for exploiting adaptive enzyme induction. Four more pairs of substances being studied are included in Table III.

TABLE III. *Other systems under investigation.*

Potentiator	Mustard
G $Ph.NH.CO.\underset{\displaystyle R'}{\underset{\vert}{CH}}.NH_2$	$R.NH.CO.\underset{\displaystyle R}{\underset{\vert}{CH}}.NH_2$
H $Ph.NH.CO.O.CH_2.COOH$	$R.NH.CO.O.CH_2.COOH$
I $Ph.O.CO.NH.CH_2COOH$	$R.O.CO.NH_2.CH_2.COOH$
J $Ph.N=N.R'$	$R.N=N.R'$

$R=(Cl.CH_2CH_2)_2N$ 

Use of Adaptive Permeases

Just as enzymes may be induced, so may permeases. The results obtained with polyols, e.g. mannitol derivatives, by several observers recently, suggest strongly that with such compounds the presence of a permease in the plasma membrane is a decisive factor. A study of permease induction made on tumour cells would be of considerable value for drug design.

Use of other Induced Processes

Many other control mechanisms are potentially available for use. For example, increased knowledge of the control of pinocytosis may make it possible to obtain selective uptake of macromolecular drugs or of antibodies. There must be a trigger mechanism for inducing rupture of lysosomes: this can probably be used to induce autolysis of tumour cells. Studies with tritiated thymidine have shown that chromosomes do not necessarily duplicate synchronously. By dissociation of the mechanisms controlling chromosome gene duplication, it may be possible to convert part of the genotype of tumour cells into the equivalent of an autogenous virus.

Dependence upon Human Biopsy

The methods which are suggested above for obtaining selectivity will usually also ensure that a given drug will act only upon a narrow spectrum of tumours. The matching of drug to tumour is not readily conducted. In a preliminary examination of about 80 human tumour specimens we have found that tumours of similar histology may differ by ten-fold in their content of a given enzyme. Thus in the selection of an appropriate drug it is likely to be essential to have available data based on examination of biopsy specimens.

A further factor calling for concern in dealing with patients is the relative sensitivity of the bone marrow. So long as mitotic poisons such as mustards are used, the bone marrow will be a limiting factor. Alternative escapes from this dilemma are available. One is to use drugs which are not mitotic poisons. A wide variety of human tumours in any case grow so slowly that little value can attach to attack on mitosis. Another is to obtain substances which will selectively protect the bone marrow. Ralph Jones has urged that the marrow cell-seeking properties of antimalarial drugs might be used in design of anti-leukemia drugs. They could equally well be used to localize protective substances in the bone marrow, e.g. in the case of an enzyme-activated drug, an appropriate enzyme inhibitor concentrated in the marrow would probably be a great asset.

CONCLUSION

From our present knowledge of cells, and of the theory of cells, an enormous range of approaches to tumour control is available. This range will inevitably increase as our knowledge of cells becomes more precise and more comprehensive.

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DISCUSSION

HEIDELBERGER: Are you sure that the acetyl groups have to be cleaved in order that the nitrogen mustard should be active?

DANIELLI: Yes.

BERGEL: Danielli has touched on the mystery of the mechanism of action of biological alkylating agents. Lawley and others have put forward the idea, based on experimental evidence mainly *in vitro*, that the mustards interfere with nucleic acids by interacting with the N^7 position of the guanine moiety to form quaternary compounds. With a derivative of myleran it appears, following the work of Roberts and Warwick, that the main points of interference with cellular constituents are SH-groups of essential peptides and proteins. There, the formation of 3-hydroxy-tetrahydrothiophene leads to a de-thiolation process altering substantially the peptides or proteins. May I ask whether mammalian tissue enzymes are inducible?

DANIELLI: We know that the amounts of enzymes can be increased, for example: those enzymes which split barbiturates, and we are investigating inducible enzymes in the Walker tumour. It may interest you to know that treatment of recurrent human mammary tumours with the urethane-potentiated mustard has given the following results; of 9 cases, 4 showed improvement and 1 had a remission lasting 6 months.

MAGASANIK: One must be careful to differentiate inducible enzymes in mammalian tissues from those which are merely stabilised by substrate. One such enzyme is thymidylate kinase, as shown by Hiatt and Bojarski (*Fed. Proc.* 1960, **19**, 309).

DE DUVE: It certainly seems that chemotherapy along the lines suggested by Danielli will really require drugs tailored to the patient's own enzymic "measurements". May I suggest that there should be some attempt to investigate the enzymic composition of a wide spectrum of human tumours? I realize that this may require action at an international level.

ELSON: Is the potentiation of action of the nitrogen mustard compounds given in Table II confined to their action on the tumour or is their toxicity and depression of circulating blood cells and of bone marrow activity also potentiated?

DANIELLI: We do know that there is increased liver damage in potentiated animals.

PETERS: I am quite sure that, in the design of drugs against tumours, it will be necessary to have the right theory of cell organization. Some years ago I put forward the idea of the "cytoskeleton" and there would now appear to be increasing evidence for it. It would be better perhaps to call this the "cytomosaic", which would include a surface as well as an endoplasmic reticulum.