the general hospital and gives results which are definitely superior to other techniques more usually available for investigating the pancreas. We think that it is suitable for use as a screening test, possibly leading to more advanced pancreatic investigations, when these seem appropriate. Useful information can be gained which excludes the pancreas from further inquiry if the scan is normal, and help provided in the differential diagnosis of malabsorption and jaundice. "False-positive" scans were not a great source of difficulty, but abnormalities may be expected in diabetes mellitus and after truncal vagotomy.

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Today's Treatment

Blood and Neoplastic Diseases

A Rational Approach to the Chemotherapy of Human Malignant Disease—I

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This article is concerned only with the rationale behind the chemotherapy of patients with advanced malignancy. I will concentrate largely on those drugs which come under the heading of cytotoxic agents or antimetabolites.

Type of Drugs

The initial choice of a drug for treating malignancy depends on its efficacy and toxicity in experimental tumours in animals. Unfortunately, there can be no foolproof method of screening these agents and undoubtedly many drugs which could have been useful in man have been missed. Nevertheless, some useful chemotherapeutic agents have been found using these methods (table I). The experimental animal chosen, the type of tumour, and the way in which the drug is administered are of paramount importance.

TABLE I—Therapeutic indices of Different Antitumour Agents*

Experimental Tumour	Agent	Therapeutic Index
Gardner lymphosarcoma	Asparaginase	7,500
Gardner lymphosarcoma	Cyclophosphamide	0-20
PC6 (plasma cell tumour)	Melphalan	†280
YOSHIDA ascites tumour	Cyclophosphamide	200
Walker tumour	Cpd 1954	70

*T. Connors, personal communication

†The highest recorded therapeutic index for a cytotoxic antitumour agent

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L-asparaginase was discovered in this way and has the highest therapeutic index of any agent in any experimental tumour system. Though L-asparaginase is effective against acute lymphoblastic leukaemia (A.L.L.) and some other lymphoid malignancies, its use has been disappointing in man in relation to its promise from experimental work. Certainly many drugs which are curative in animals may have little effect in man, and for this reason a clinical trial is an important second step. After this, comparative trials may be set up to assess the value of different single agents. Any clinical evaluation of a new drug involves three steps: firstly, pharmacological evaluation to establish toxicity (antitumour activity not required); secondly, measurement of clinical activity against several tumours; and thirdly, comparative studies to establish its role in the treatment of a tumour. This involves comparison with the best standard treatment and the evaluation of combined treatments.

Some of the more important agents which have proved to be of value in human cancer are classified in table II. The mech-

TABLE II—Chemotherapeutic Agents in Common Use

Mechanism	Class	Examples
Interfere with DNA synthesis	Pyrimidine analogues Purine analogues Folate antagonists Hydroxyurea	Cytosine Arabinoside 5-Fluorouracil 6-Mercaptopurine 6-Thioguanine Methotrexate
Interfere with mitotic spindle formation	Vinca alkaloids	Vincristine vinblastine
Alkylate and cross link strands of DNA at any stage of cell cycle	Alkylating Agents	Mustine hydrochloride Cyclophosphamide Busulphan Chlorambucil Danorubicin Duoxymycin (Adriamycin)
Bind with DNA and interfere with replication	Antibiotics	Adriamycin (Cerbubidin) BCNU CCNU Me CCNU
Action on transfer RNA	Actinomycin D	
Action unknown	Nitrosoureas Procarbazine	

anism of action of any individual drug has nearly always been determined after its efficacy has been shown, but there are a few examples where a rational approach has led to the development of a new agent. One instance was the development of 5-fluorouracil after the observation that certain tumours preferentially take up uracil compared with normal tissue.¹ Nearly always the approach has been empirical. Some agents are highly specific for a particular tumour—for example, corticosteroids in the treatment of lymphoid cell tumours, and O, p. DDD (mitotane) on cells of the adrenal cortex.

Nearly all cytotoxic drugs in current use act by interfering with cell replication. Some, such as methotrexate, cytosine arabinoside, and the vinca alkaloids are "phase dependent" and act either during the DNA synthetic phase (S-phase) of cell growth, or during mitosis. Others, such as the alkylating agents, are "phase independent" and can affect cells at any stage in the cell cycle, though usually they still have more effect during the S-phase. Figure 1 shows the cell cycle in diagrammatic form.

FIG. 1—Cell cycle.

Combination Chemotherapy

It is possible to devise a combination of drugs which have a different mechanism of action and provide greater benefit than the single agents do individually. If the side effects of the drugs are different these will not be additive, and the combination need be no more toxic than the agents used singly. In some experimental systems it can be shown that the benefit produced by a combination of two drugs can be greater than the additive effect of the agents used singly. Table III gives an example in

TABLE III—*Synergistic Antitumour Action of Melphalan and Methotrexate vs. NK/Lymphoma*

Agent	Dose (mg/kg)	% Inhibition
Melphalan (Single dose)	8	40
	4	35
	2	20
	1	Nil
Methotrexate (daily for five days)	10	34
	5	10
	2.5	Nil
Melphalan (single dose) and Methotrexate Daily (2.5 mg/kg for five days)	8	100 ("cures")
	4	59
	2	24
	1	Nil

*J. A. Double, Unpublished results, 1969.

TABLE IV—*Showing that a Suitable Combination of Drugs may Improve the Remission rate over the Same Drugs Used Singly*

Chemotherapy Single agent	Complete Remission Rate		
	Hodgkin's Disease 25%	Acute Lymphoblastic Leukaemia 50%	Acute Myelogenous Leukaemia 25%
Two agents	63%	85%	50%
Three or more agents	75%	95%	50%

which melphalan alone gives 40% inhibition of tumour growth, but methotrexate administered daily at a dose of 2.5 mg/kg gives no significant inhibition. The combination of the two drugs, however, cures the lymphoma. This phenomenon is known as synergism.

There are now several examples in man where a combination of drugs has proved of greater benefit than the same agents used singly. This is true, for example, in remission induction in Hodgkin's disease, A.L.L., and acute myelogenous leukaemia (A.M.L.) (table IV). Drugs such as procarbazine, an alkylating agent, or a vinca alkaloid used alone, produce complete remissions in about 25% of patients with Hodgkin's disease. With vinblastine together with chlorambucil complete remissions have been achieved in 63%, but with a combination of mustine hydrochloride, procarbazine, prednisolone, and a vinca alkaloid, this figure rises to 75% or more. The introduction of combinations of cytosine arabinoside with daunorubicin or thioguanine in patients with A.M.L. has increased the complete remission rate from less than 25% to about 50%. Vincristine or prednisolone used alone for the treatment of A.L.L. in childhood will produce remissions in 50-60%, but a combination of the two leads to remissions of more than 85%. The addition of daunorubicin has further increased the remission rate, but similar results are obtained if the daunorubicin is given after the vincristine and prednisolone.

ANTAGONISTIC COMBINATIONS

A word of caution is required about the use of combination of drugs in human cancer, since not all combinations in animal systems are advantageous: some are antagonistic. For example, a combination of methotrexate and L-asparaginase or methotrexate and cytosine arabinoside have proved disadvantageous in some experimental tumours. Possibly this is because methotrexate kills cells by inhibiting thymidylate synthesis but, in addition, has some inhibition of purine synthesis which protects some cells by preventing DNA synthesis. A combination of methotrexate and a pure DNA inhibitor such as cytosine arabinoside, would not be curative, since some cells would be held in G₁ (see fig. 1).

Another form of combination of drugs which may prove to be of value involves the property of one drug to reduce the detoxification of a chemotherapeutic agent. Allopurinol, for example, will greatly enhance the effect of 6-MP by inhibiting enzymes concerned in the breakdown of this drug. Recovery from the effects of an alkylating agent depends on adequate DNA repair. Some drugs, such as the antimalarial agents quinacrine and chloroquine, inhibit enzymes concerned in DNA repair and possibly this effect could be used to increase the cytotoxic effect of a chemotherapeutic agent. Membrane repair may also be a factor in the survival of a damaged tumour cell. Several repair mechanisms are being actively investigated in the hope that they will produce methods for increasing drug susceptibility of tumour cells.

A new concept of using drugs complexed with DNA has recently been introduced.² A daunorubicin-DNA complex appears to be less toxic than the unbound daunorubicin and the hope is that it may prove to be clinically more useful. The cytotoxic action is realized only within the cell when the complex is enzymatically disrupted. The drug is expected to have a greater effect on cancer cells, because of their high lysosomal content and increased ability to cope with the complex by pinocytosis compared with many normal tissues. Another type of compound which has been developed is a drug-antibody complex which is hoped to home specifically on the tumour cells.³ Clinical trials are underway to assess the value of such complexes, and preliminary results suggest that they may be beneficial.

Agents such as barbiturates which increase hepatic enzyme activity may appreciably affect the metabolism and effect of a cytotoxic drug—for example, cyclophosphamide in experimenta

animal tumours. The chemotherapist has been slow to realize the possible importance of common drugs which alter protein binding or increase liver detoxifying activity. Little is known about drug interactions of this sort in man, but it is becoming clear that these considerations will play an important part in the more precise use of cytotoxic drugs in the future.

Route of Administration

The route by which a drug is administered may be extremely important in experimental systems, and may make the difference between cure of a tumour and complete failure (table V). Thus intraperitoneal or intraportal injection of mustine hydrochloride was found to have less than one quarter of the effect on the Walker tumour than the same amount of drug given intravenously.⁴

TABLE V—Influence of Route of Administration on the Antitumour Activity of Mustine Hydrochloride on the Walker Tumour

Intravenous		Intraportal	
Tumour Curative Dose	LD ₅₀	Tumour Curative Dose	LD ₅₀
0.1 mg	1.5 mg	None	1.8 mg

after Cobb⁴

In man cytotoxic drugs are frequently given by mouth, and most of the absorbed material passes through the liver before reaching the tumour. Detoxification frequently occurs in the liver and this will reduce the effective concentration reaching the tumours. In addition, intestinal absorption normally varies a great deal, and as little as 20% of the dose may be absorbed. Nausea and vomiting with many of the drugs may lead to little being absorbed.

Drug Sequence

The sequence in which two drugs are given may have an important effect on the response rate. For example, a hamster plasmacytoma cannot be cured with a potent cycle-active agent (cytosine arabinoside) when the tumour is extensive, but it can be cured with the same drug if the total mass is first reduced by several logs with cyclophosphamide (reversal of this sequence is ineffective). Though the drug sequence may have an important effect in animal tumours, the role of drug sequence in human tumours is unknown; nevertheless, it is likely to be important. A consideration of tumour cell proliferation rates can provide some explanation of these results.

Tumour Cell Proliferation Studies

The concept that cancer is a group of diseases in which a rapidly proliferating, autonomous population of abnormal cells, overruns the normal tissues is quite wrong. In acute leukaemia, for example, the blast cells have generation tissues that are longer on average than normal bone marrow cells, and leukaemic myeloblasts divide more slowly than normal myeloblasts.⁵

In malignant disease, however, the cells gradually tend to accumulate because the cell loss is not 100%. Normal myeloblasts mature into polymorphs and are eventually lost to the body, and there is no net increase in cell number. The proliferation of a tumour is not entirely autonomous, neither is it constant for it varies with the size of the tumour and is related to its blood supply.

Animal studies show that the characteristics of tumour cell proliferation have an important influence on the response to chemotherapy. Skipper⁶ has reviewed some of the principles concerned. The following generalizations may be stated:

(1) The doubling time of a tumour increases with the tumour mass and the thymidine-labelling index decreases (table VI).

TABLE VI—Mass of Tumour and Doubling Time

Days Post Inplant	Average Mass (mg)	Doubling Time (days)
8	220	1.2
16	3400	7.4
20	5200	>10

Carcinoma 755 system.⁶ Tumours induced by fragments taken from small, rapidly growing tumours and from large slowly growing tumours grow at the same rates.

(2) The response to chemotherapy is proportional to the thymidine-labelling index (for example, the number of cells synthesizing DNA).

(3) The shorter the doubling time at the onset of treatment, the better the response to chemotherapy.

(4) As the tumour grows the disease becomes less easy to cure.

(5) Alkylating agents are generally more effective than S-phase-specific drugs against tumours with longer doubling times and lower thymidine-labelling indices.

Though the data are more sparse for human tumours, there are suggestions that these generalizations also apply to human cancer. Tumours such as gestational choriocarcinoma, Burkitt's lymphoma, and the acute leukaemias have relatively large growth fractions and high thymidine-labelling indices. These diseases respond much better to chemotherapy than tumours with smaller growth fractions, such as carcinoma of the lung and gastrointestinal tract. If the generalizations apply to human cancer, trials of drugs in human cancer using these concepts will be very important in the future. It may, for example, be possible to apply to human myeloma the techniques described previously in the hamster plasmacytoma system. The growth rate of human myeloma gradually falls as the tumour cell number increases.⁷ Figure 2 shows that both the growth and regression of myeloma cells are non-linear. With large amounts of tumour at the time of diagnosis the thymidine-labelling index is low, and the best chance of a useful clinical effect would be to start treatment with an alkylating agent to reduce the tumour cell population and then treat with phase-dependent agents such as cytosine arabinoside and vincristine at a time when the thymidine labelling index is expected to be increased (see fig. 3).

To carry these concepts to a logical conclusion, it is reasonable to suggest intensive treatment during remission with phase-dependent agents (cf. acute leukaemia treatment) for all tumours in which complete remissions can be achieved, the residual tumour cell number is small, and the growth rate is expected to be high. Experimental studies show that the effect of an antitumour agent on tumour cells follows first-order kinetics. This means that for a given treatment the fractional reduction is constant and not the absolute reduction of tumour cells. This also supports the concept that chemotherapy has

FIG. 2—Log of tumour cell number vs. linear plot of time in an untreated case (a) and a treated case (b). Neither growth nor regression is linear (From Sullivan and Salmon⁷).

FIG. 3—Kinetics of response to chemotherapy.¹⁰

a maximum chance of being curative when fewer tumours cells are present. Controlled trials in man are necessary to test these ideas. Some, such as those for myeloma are already in progress.

Recent information suggests that combined chemotherapy for inapparent residual disease can be highly effective. Long remissions following primary surgical treatment for osteogenic sarcoma are now being reported using intensive combination chemotherapy. A similar approach in patients with Wilm's tumours has also been efficacious. Early reports suggest that this form of adjuvant chemotherapy may also be of advantage in the more common cancers such as breast carcinoma. Undoubtedly further trials will be reported in the near future on the role of adjuvant chemotherapy in several common cancers which have a high incidence of death from metastatic disease following primary treatment.

Timing of Drug Administration

The time at which drugs are given has an important influence on toxicity, and in the case of mouse leukaemia, may make a difference between success and failure of therapeutic regimen (table VII).

In this experiment, it was shown that there is an optimum interval between injections of an S-phase-specific agent. The same drug given at other times, at up to twice the total dose, was attended by uniform failure. Hopefully, drugs will be found which will enhance a transition of cells from G₁ or G₀ into S-phase. This would be of particular advantage in tumours with small growth fractions. Attempts are being made in man to alter the timing of administration of cytotoxic

TABLE VII—Timing of Ara. C and Response of L1210 Leukaemia

Mg/Kg/dose	No. of Doses	Interval Between Doses	Interval Between Doses during which Blood Concentration was <0.3 µg/ml (hr)	% Cures with 10 ⁴ Cells
15	8	10 min—1 hr	0	0
15	8	3 hr	1	60
15	8	8 hr	6	30
15	8	9 hr	7	0

Modified table from Skipper.⁸

drugs in such a way as to increase the effect on leukaemic cells and reduce the effect on normal cells. This effect may be increased by attempting to synchronize division of the leukaemic cells with cytotoxic drugs. The number of leukaemic cells in S-phase can be increased by treatment with a single agent such as cytosine arabinoside.⁸ It is to be expected that if the proportion of cells in S-phase is increased tenfold, then the administration of an S-phase-specific agent at the optimum time may result in a ten-fold greater effect on the leukaemic cell population. If the normal bone marrow population has a different growth cycle the toxicity could also be minimized.

Cytotoxic drugs are usually used intermittently to allow regrowth of normal marrow and gut epithelium. In this way the toxicity is reduced and, in addition, the efficacy may be increased. A clear example of this is the use of intermittent methotrexate during remission in A.L.L. Twice-weekly methotrexate is clearly more effective than a daily dose schedule in maintaining remission.⁹ The methotrexate dose was adjusted to the limits of toxicity in both schedules.

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Any Questions

We publish below a selection of questions and answers of general interest

Signs of Protein Deficiency

What are the early signs of protein deficiency?

Dietary protein deficiency is very rare in western countries. The amounts of protein required by an adult are much less than had previously been thought. No more than about 40 g a day is sufficient, and it is difficult to devise a diet that is adequate in calories but deficient in protein. For this reason dietary protein deficiency also seems to be rare in adults in poorer countries. But some protein deficiency is common in babies in these countries and is often associated with a calorie deficiency. This is partly because young children require a higher proportion of protein in their diet and partly because they tend to be given less of the protein-rich foods that may be available. Protein-deficiency disease is kwashiorkor and the first signs are a failure to grow, wasted muscles, apathy, and oedema. Deficiency may arise from non-dietary or secondary causes, such as liver or kidney disease, severe trauma such as burns, or malabsorption. It shows itself in a

loss of weight, accompanied by a loss of nitrogen, a decrease in tissue mass, and a fall in plasma albumin concentration.

Simultaneous Use of Lithium with Tranquillizers

Is there any danger in continuing lithium therapy with tranquillizers or antidepressant drugs in the treatment of depression?

There is no danger in continuing lithium therapy with tranquillizers or antidepressants provided the doses of each drug used are appropriate and there are no unwanted or toxic effects. The use of lithium carbonate does not increase the tendency to side effects from antidepressants or tranquillizers, and this is of value in the treatment of recurrent depressive states. As the prophylactic effects of lithium carbonate may take up to 18 months to appear, treatment with antidepressants which were previously helpful can be given in the interim should a depressive phase develop.