Review Letter

# DRUGS USED IN THE TREATMENT OF CANCER

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Received 4 July 1975

## 1. Introduction

There are many types of cancer, some occurring commonly and others which are very rare. Different cancers can be distinghuished from one another because of differences in their morphology and natural history, in their cell kinetic and biochemical properties and from their response to chemotherapy.

At one time, chemotherapy was used primarily in the treatment of leukaemias and lymphomas, and in a few other types of cancer such as choriocarcinoma [1]. More recently it has been shown that chemotherapy given immediately after surgery or radiotherapy (termed adjuvant chemotherapy) has significantly increased the proportion of long-term survivors in other types of cancer and is currently being tested in many more. Preliminary results using simple (one drug) adjuvant chemotherapy of breast cancer, a very common cancer, has indicated that a significant reduction in recurrence of the cancer may be obtained if the drug is given soon after surgery [2]. Since a substantial proportion (30%) of operable breast cancers recur after surgery, small deposits of tumour must have been left behind in many cases, usually as metastases at a distance from the primary tumour. The aim of adjuvant chemotherapy is to treat the metastases when they consist of only a small number of cells, at which time they are most susceptible to chemotherapy.

Progress in cancer chemotherapy is relatively slow, but once a cancer is known to be sensitive to one or more chemotherapeutic agents, results usually get progressively better as the optimal conditions for chemotherapy are worked out by clinical trials. In 1960, for example, although the first anti-cancer agents had been discovered and were undergoing clinical evaluation, survival from acute lymphoblastic leukaemia of childhood was measured in terms of months. In 1967, the proportion of children (in the United States) treated by chemotherapy and surviving longer than five years without recurrence of the disease was 25%, and by 1972, this proportion had risen to 50%. Since it is expected that most patients will never relapse after having been free from tumour for five years, then it is clear that a cancer which was incurable 15 years ago can now be successfully treated by chemotherapy in a large proportion of cases.

Advances in chemotherapy have been made by the discovery of new classes of anti-cancer agents [3], by the use of drug combinations [4], and by learning more about the cell kinetic and biochemical properties of tumours and the way in which they differ from normal tissues [5,6]. This article is restricted to a description of the types of chemical that have anti-cancer activity and the ways in which attempts are made to improve upon their selectivity.

## 2. Cytotoxic agents

Anti-cancer agents used today are not specifically toxic for cancer cells in the same way that the sulphonamides, for example, are selectively toxic for bacteria. The basic property of anti-cancer agents in their ability to kill dividing cells in general and thus they can prevent tumour growth but only at a cost to normal dividing tissues such as bone marrow and mucosal surfaces. For this reason anti-cancer agents are referred to as cytotoxic agents but under certain conditions complete tumour cell destruction can be achieved at dose levels which do not cause unacceptable toxicity.

Anti-cancer agents may be classified according to the stage of the cell cycle at which they act or, more

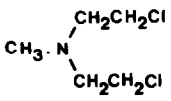
usually, according to their chemical structure and mechanism of action. By the latter classification, there are (a) a large class of electrophilic reactants, (b) the anti-tumour antibiotics, (c) the antimetabolites, particularly the anti-folates and the purine and pyrimidine antagonists, and (d) a variety of chemicals which arrest cells in mitosis and are referred to as the spindle poisons. In addition, there are several agents which do not belong to any of these classes.

## 3. Electrophilic reactants

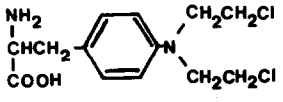
### 3.1. *Bifunctional alkylating agents*

The aliphatic nitrogen mustard HN2 (I) was one of the first anti-cancer agents to be used effectively in the clinic and since that time many more related alkylating agents have been studied. Clinically usuful compounds include the aromatic nitrogen mustard, melphalan (II), the bisulphonoxyalkane myleran (III), and the ethyleneimide, thioTEPA (IV). All act in a similar way by alkylation of nucleophilic sites in cells, for example ionised acidic and thiol groups and uncharged amine groups of nucleic acids and proteins. Usually the alkylating agents act by an $S_N2$ mechanism, involving the formation of an intermediate transition complex, but the less basic aromatic nitrogen mustards can act by an $S_N1$ mechanism, where the rate-limiting step is ionisation to form a carbonium ion. The bifunctional alkylating agents all have similar distinctive biological properties. Since a tumour sensitive to one alkylating agent is likely to be sensitive to another, and because tumours with acquired resistance to one alkylating agent are cross-resistant to all others, it is assumed that all bifunctional alkylating agents act by a similar mechanism. The absolute requirement for at least two alkylating entities in the molecule suggested that the cytotoxicity of the alkylating agents was due to a cross-linking reaction involving alkylation of two nucleophilic centres in the same or different molecules. Because the alkylating agents react with many biological molecules, it has proved difficult to determine the exact mechanism by which they kill cells. The best hypothesis at present is that the most sensitive site in the cell, alkylation of which leads to toxicity, is DNA and that some form of inter- or intra-strand cross-linking takes place. In particular situations, however, it has been claimed that glycolytic pathways, cell membranes, nucleoprotein and cAMP phosphodiesterase are all essential target sites for the alkylating agents.

Although it is likely that all alkylating agents act in the same way, some may be more effective than others as anti-cancer agents. Whether or not a drug will be active against a particular cacer will depend on the balance between the damage caused to the cancer and the damage to normal tissues. The biochemical and cell kinetic properties of a cancer, as well as its location in the body and its blood supply, are just some of the features which will determine its sensitivity to chemotherapy. The tissue distribution of a drug, its biological and chemical half-life, its differential metabolism in normal and tumour cell, and whether any damage it causes can be repaired, are all factors regulating the degree of anti-tumour effect attainable under optimal conditions.



(I)



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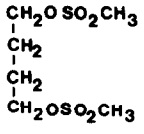
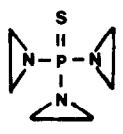


(III)

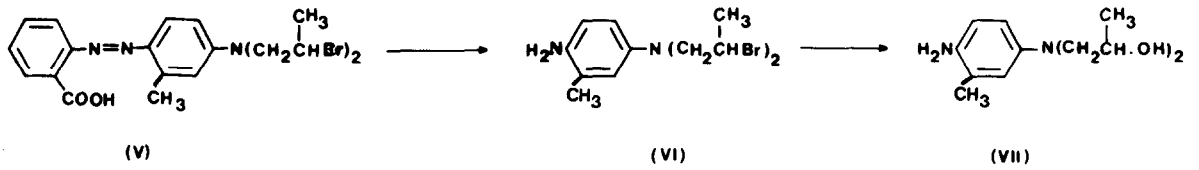


(IV)

### 3.2. *Mechanisms of selectivity*

Alkylating agents usually enter cells by passive diffusion, but HN2 [1] is actively transported by some cells utilising the choline transfer system [7], which may explain why it is the preferred alkylating agent in the treatment of some forms of cancer.

Agents have also been designed whose chemical half-lives are extremely short [8]. These are injected directly into the blood vessels supplying the tumour, which is therefore exposed to a high concentration of alkylating agent. Most of the alkylating agent escaping from the tumour will have reacted before it reaches the bone marrow. This approach has had some success in the treatment of head and neck cancer, but where tumours are localised and readily accessible they are

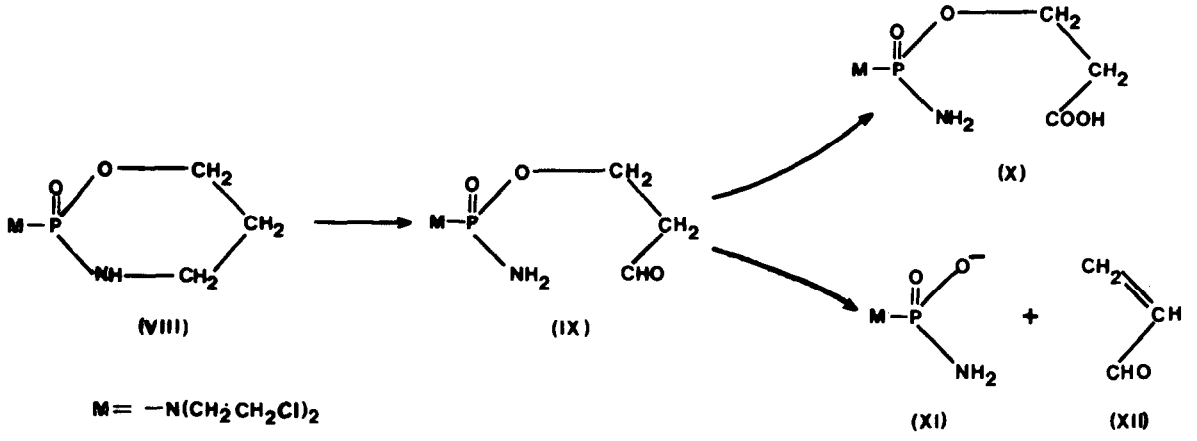


(V) (VI) (VII)

usually better dealt with by surgery or radiotherapy.

Enzymes that occur in very high concentration in cancer cells can also be the basis for the design of selective agents. Thus, primary liver cancer has a high level of azo-reductase which can convert a noncytotoxic azo-mustard (V) into a chemically reactive and highly cytotoxic amine (VI) whose half-life is so short that any of it that leaves the liver will have reacted harmlessly in the extracellular fluids or have been hydrolysed (VII) before reaching the bone marrow [9] There will also be a high livel of alkylation of normal liver cells but these are mostly non-dividing and non-sensitive to alkylation. A similar approach uses aniline mustard which is converted to its para-*O*-glucuronide in liver and then hydrolysed to the highly toxic para-hydroxyaniline mustard in tumour cells with exceptionally high glucuronidase levels. Unfortunately, although particular animal tumours may be found to have abnormally high levels of an enzyme, it does not follow that there will be human tumours with similarly high levels of the same enzyme. A significant advance in this field has been the demonstration that human tumours can be grown in mice whose immunological responses are depressed genetically ('nude' athymic mice) or artifically (T lymphocyte-deprived mice) [10]. As these tumour models begin to be used in experimental chemotherapy, they may be found to have unusual concentrations of enzymes which may be characteristic of a particular type of human cancer and thus provide the basis for rational chemotherapy.

The absence of enzymes in cancer cells may also make them susceptible to chemotherapy. Cyclophosphamide (VIII) is, in many cases, a better anti-cancer agent than related nitrogen mustards. Although acting by the same mechanism, cyclophosamide is highly selective, probably because it is hydroxylated in the liver to 4-hydroxycyclophosphamide, which is in equilibrium with its tautomeric aldehyde (aldophosphamide; IX). Neither (VIII) nor (IX) are chemically reactive and the latter can be converted by aldehyde dehydrogenases to the propionic acid (X) which is also noncytotoxic. In the absence of any enzymes, however, (IX) can undergo a chemical $\beta$-elimination to form phosphoramide mustard (XI) and acrolein (XII), both of which are cytotoxic. It has been suggested that the higher selectivity of cyclo phosphamide compared with other alkylating agents can be accounted for by the greater breakdown of aldophosphamide in tumour cells to phosphoramide mustard because of their lower levels of dehydrogenase enzymes [11,12].

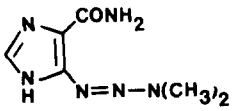


(VIII) (IX) (X) (XI) (XII)

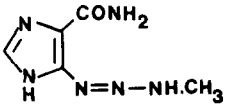
M= $-N(CH_2CH_2Cl)_2$

### 3.2. *Other electrophilic reactants*

Dimethyltriazenoimidazole carboxamide (DIC; XIII) is believed to act by some form of alkylation, but it is clearly different from bifunctional alkylating agents in its mechanism of action. It can, for example, cause complete regression of animal tumours which are resistant to alkylating agents. Inactive in its own right, DIC is metabolised by liver microsomes to the monomethyl derivative (XIV) which can react directly with nucleophiles or which can break down to alkylating fragments such as methyl carbonium ions. One of the problems associated with the clinical use of DIC is that it causes serious nausea and vomiting. Since it can break



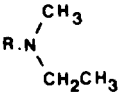
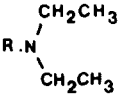
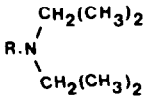
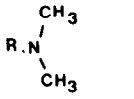
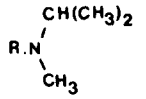
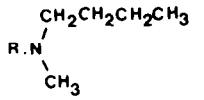
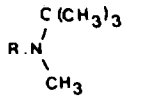

(XIII)




(XIV)

Table 1

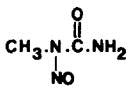
Activation of phenyltriazenes. Only those alkytriazenes that are metabolised by liver microsomes in vitro to 3-*N*-methylphenyltriazene have activity against the mouse TLX5 transplanted lymphoma. Alkyltriazenes that do not form this metabolite are inactive, despite the fact that they can also break down to alkylating moieties.

| Agent | Probable Microsomal Metabolite | Anti-tumour Activity |
|---|---|---|
|  (XV) | R.NH.$CH_3$ | + |
|  (XVI) | R.NH.$CH_2CH_3$ | – |
|  (XVII) | R.NH.$CH_2(CH_3)_2$ | – |
|  (XVIII) | R.NH.$CH_3$ | + |
|  (XIX) | R.NH.$CH_3$ | + |
|  (XX) | R.NH.$CH_3$ | + |
|  (XXI) | R.NH.$C(CH_3)_3$ | – |

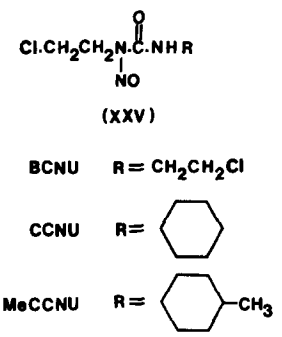
down by photochemical reaction to a diazonium compound which is higly toxic and could be the cause of the side effects, a series of phenyltriazenes which are light stable, have also been investigated [13]. Table 1 shows the structure activity relationship of this series. Only chemicals that can be converted enzymatically to phenyl methyltriazene have antitumour properties. Thus the methylethyl analogue (XV) is active because de-ethylation is preferred to demethylation and the active monomethyltriazene is formed. Although the diethyl analogue (XVI) is dealkylated as readily as the dimethyl analogue (XVII), it is clearly inactive. The mixed methylalkyltriazenes (XIX and XX) are both active and, since no demethylation could be detected on incubation with microsomes, it was assumed that the higher alkyl group was being removed. This cannot occur with the tertiary isobutylmethylphenyltriazene (XXI) since the higher alkyl group has no $\alpha$-hydrogen and this compound is readily demethylated by liver microsomes. In this case, the tertiary monoisobutylphenyltriazene is formed and (XXI) is, as expected, not active as an anti-tumour agent. The selective anti-tumour effect of monomethyltriazenes compared to higher alkyl derivatives has not yet been explained, but it is possible that, if they are electrophilic reactants in their own right, their essential target site in the tumour cell is sterically hindered and only accesible to monomethyltriazenes.

A requirement for an N-methyl rather than an N-ethyl or higher alkyl group is also seen with procarbazine (natulan; XXII). Like the triazenes, procarbazine is not active itself, but decomposes in vivo into as yet unidentified anti-tumour agents [14,15].

Another N-methyl derivative which has anti-cancer activity is hexamethylmelamine (HMM; XXIII). Although almost rejected because of its minimal activity against animal tumours, HMM has significant activity in man, particularly in the treatment of lung and ovarian cancer. It is also highly active against human tumours transplanted into immunologically-deprived mice, especially lung [16] and kidney tumours, but also against tumours of the ovary and colon. Little is known of the mode of action of HMM, but it is reminiscent of the triazenes in that small changes in structure can lead to complete loss of activity (Table 2). There is an apparent correlation between demethylation and anti-cancer activity (Table 2) and this has led to the suggestion that it is an intermediate methylol which may be the active metabolite. N-Methylols have been postulated as cross-linking agents responsible for the mutagenicity of formaldehyde [17].

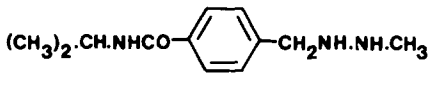


(XXIV)

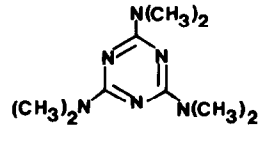



(XXV)

Methylnitrosourea (XXIV), although a potent carcinogen, is also used in the treatment of lung cancer. Its anti-tumour properties were first demonstrated many years ago and a series of related chloroethylnitrosoureas based on this structure have since been used clinically (BCNU, CCNU, MeCCNU; XXV). They decompose rapidly under physiological conditions, to a number of carbamoylating and alkylating products as well as aldehydes or alcohols [18]. Cytotoxicity may be the combined result of a variety of reactions, e.g. interferences with the function of DNA by alkylation and inhibition of DNA alkylation repair enzymes by carbomoylation. No explanation can yet be given for their selective anti-tumour effects which, in the case of some animal tumours, is quite remarkable. For instance, the TLX5 lymphoma, in CBA/LAC mice, grows from a single cell. After injection of $10^6$ cells, there is a rapid spread of tumour and about 9 days later the mice die, at which time tumour cells can be found in most body organs. BCNU is so selective that tumour-bearing animals can still be treated on day 8 and a proportion of them cured.



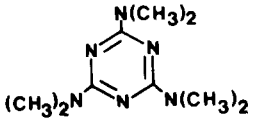
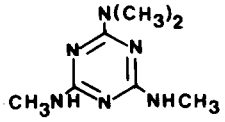
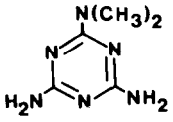
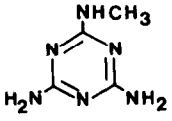
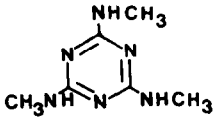
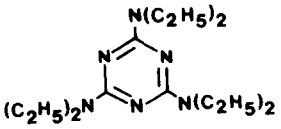
(XXII)



(XXIII)

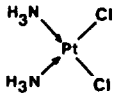
Table 2

Structure activity relationships of a series of N-substituted melamines. Only compounds that are demethylated by liver microsomes in vitro have activity against the mouse plasma cell tumour. (Expressed as a therapeutic index). The more readily a derivative is demethylated the higher is its therapeutic index.

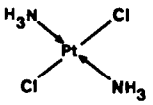
| Agent | Demethylation by microsomes | Antitumour activity |
|---|---|---|
|  | + + + | 10.3 |
|  | + | 1.8 |
|  | – | Inactive |
|  | – | Inactive |
|  | – | Inactive |
|  | | Inactive |

## 4. Platinum co-ordination complexes

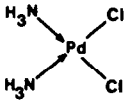
Closely related platinum complexes vary widely in their anti-cancer properties. Thus *cis*-dichlorodiammine platinum(II) (Cis Pt(II); XXVI), isolated as one of the stable bacteriostatic products formed when an electrical current was passed through a medium in which bacteria were growing, has a wide spectrum of action against animal tumours. However. the *trans* analogue (XXVII) is inactive and platinum cannot generally be replaced by other heavy metals such as palladium (XXVIII), although recently a few rhodium, iridium and palladium complexes have been shown to have anti-cancer activity [19]. Even in a homologous series, the selectivity of the compounds can vary considerably either by alteration of the tumour inhibitory dose or the lethal dose (Table 3). However, whether a compound is better than a related analogue is a property not only of the drug but of the tumour used to assess activity. The L1210 leukaemia (Table 3) appears to be particularly responsive to hydrophilic derivatives and the plasma cell tumour to lipophilic ones.



(XXVI)



(XXVII)

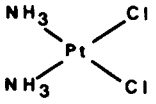
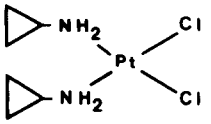
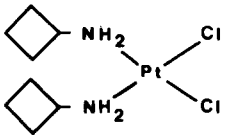
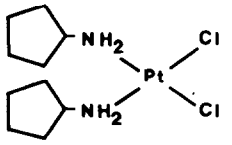
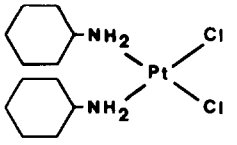


(XXVIII)

In many of their biological properties, the platinum complexes resemble the bifunctional alkylating agents, and they probably act similarly by cross-linking DNA. In this case, the most probable cross-linking site involves

Table 3

The effect of a series of platinum compounds against the mouse plasma cell tumour (PC6) and L1210 leukaemia (L1210). With increasing size of the alicyclic ring the therapeutic index (TI) against the plasma cell tumour increases. Conversely, the effect against the L1210 leukaemia, expressed as the maximum increase in life span (%ILS) at the optimum dose, decreases with increasing size of the alicyclic ring. PC, octanol water partition coefficient. Water solubility expressed as mg/litre.

| Agent | PC6 (T.I.) | L1210 (% I.L.S.) | Sol. Water | PC |
|---|---|---|---|---|
|  | 8·1 | 95 | 2,650 | <·01 |
|  | 24·6 | 70 | 610 | <·01 |
|  | 31·0 | 52 | 87 | ·05 |
|  | 235 | 41 | 5·6 | ·86 |
|  | >267 | 3 | 1·9 | 4·3 |

the amino groups of adenosine residues, especially where they lie one above the other in the same strand [19]. Despite this similarity, Cis Pt(II) (XXVI) is active in the clinic against cancers of the ovary that have acquired resistance to alkylating agents.

## 5. Anti-tumour antibiotics

As a result of screening extracts of soil micro-organisms, many different classes of antibiotics have been shown to have anti-cancer properties. This usually means they are toxic to cancer cells in culture or have some effects on the growth of transplanted animal tumours and does not necessarily mean they are useful in man. In practice, only actinomycin [20], daunomycin, adriamycin and bleomycin [21] are used in the clinic to any great extent. Bleomycin, which is a mixture of related compounds, has a particular affinity for squamous cell carcinoma and etpihelial tissues in general. Thus, the antibiotic can inhibit squanous cell carcinoma in mice induced by 20-methylcholanthrene but does not inhibit sarcoma induced in mouse skin by the same chemical. This tissue selectivity is in part due to the selective concentration of bleomycin by epithelial tissues (and lung) and in part due to their lowered ability compared to other tissues to convert the antibiotic enzymatically to a non-cytotoxic form. In contrast, daunomycin is reduced enzymatically to daunomycinol, which is probably a more selective anti-tumour agent, since anti-tumour effectiveness has been correlated with the levels of the reductase enzyme.

Bleomycin, probably because it is a mixture of products, has a variety of actions causing single-strand breaks of DNA, inhibition of DNA-dependent DNA polymerase and arrest of cells just prior to mitosis.

Adriamycin, daunomycin and actinomycin act by some form of intercalation, a molecule of the antibiotic inserting between stacked base pairs of the double helix, interfering with replication and transcription. Adriamycin is of particular interest because it has activity against solid tumours, but because it causes cardiac toxicity there are many clinical situations where it cannot be used. Its structural relationship to the cardiac glycosides has been suggested as the reason for its specific effects on cardiac muscle, and attempts are at present being made to design derivatives which are less related in chemical structure to the digitalis glycosides.

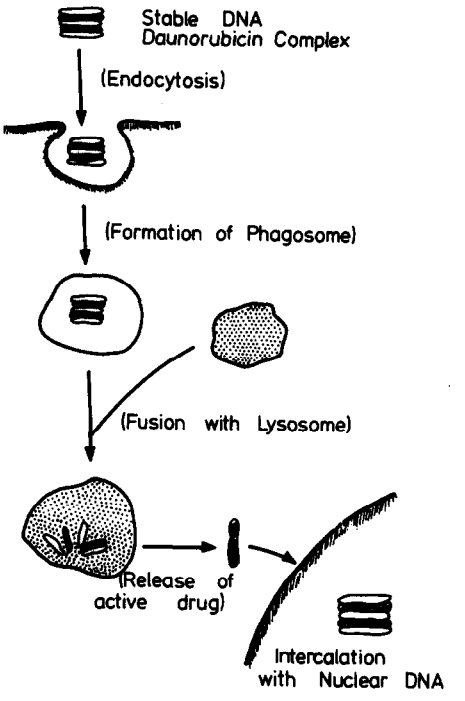


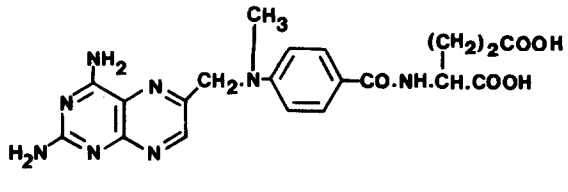

Fig.1. Use of stable DNA-Daunorubicin (or adriamycin) complexes to increase selectivity. Concentration of the complex inside tumour cells may occur by endocytosis. The resultant phagosome can condense with a lysosome and release active antibiotic inside the cytoplasm of the tumour cell. After Trouet et al. [22].

In further attempts to improve the selectivity of adriamycin and daunomycin the antibiotics have been intercalated with fragments of DNA in vitro. The resulting complexes are stable and non-toxic, but could give selectivity by the scheme of fig.1. Tumour cells are known to take up high molecular weight materials by endocytosis and thus they may, unlike cardiac muscle cells, concentrate a significant amount of the antibiotic-DNA complex. Once inside the cells (in the structure of a phagosome) condensation with a lysosome takes place and the complex is exposed to DNAase. This releases the antibiotic in its cytotoxic form inside the tumour cell [22]. These complexes are more selective anti-tumour agents than the antibiotic alone under optimal laboratory conditions, and they are at present on clinical trial.

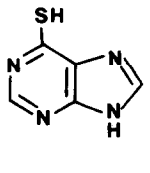
Related attempts to deliver cytotoxic drugs specifically to tumours involve attachment of cytotoxic groups to tumour 'specific' proteins which will concentrate in the area of the tumour [23] and incorporation of the drug into liposomes, which like the DNA complexes may be selectively endocytosed by tumour cells [24].

## 6. Antimetabolites

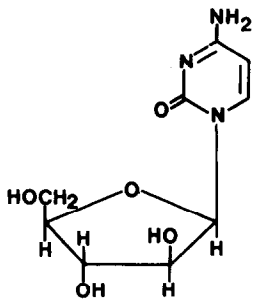
Clinically useful antimetabolites are antagonists of folic acid (e.g. methotrexate, XXIX) of purines (e.g. 6 mercaptopurine; XXX) or pyrimidines (e.g. cytosine arabinoside; XXXI. By definition, these agents substitute for normal metabolites in various biochemical pathways and their cytotoxicity is greatly influenced by the levels of natural meatbolites with which they are competing. Thus the cytotoxicity of methotrexate can be reversed by folinic acid (which bypasses the site of methotrexate inhibition) or by thymidine (the major effect of methotrexate on dividing cells is to cause a lack of thymidylate). By carefully varying the time at which cells are 'rescued' from the cytotoxicity of antifolates, an optimum situation can be found where normal tissues can be rescued but at a time when cancer cells are already irreversibly inhibited [25]. Large numbers of new types of anti-metabolite have been investigated and a new one of interest is 5-azacytidine (XXXII) which is not cross-resistant to cytosine arabinoside in patients with acute granulocytic leukaemia. Cytosine arabinoside is limited in its clinical use because it must be maintained at a physiologically effective dose in the body for the period of time that it takes all cancer cells to enter the phase of the cell cycle (DNA synthesis) which is sensitive to the drug. Because cytosine arabinoside is rapidly broken down by cytidine deaminase in the liver and other organs, these levels can only be maintained by infusion. More stable derivatives acting in the same way are currently being investigated, such as cyclocytidine (XXXIII), which has a long biological half-life and slowly hydrolyses to cytosine arabinoside [26]. A novel approach has been to synthesis transtion state analogues [27]. Thus carbamyl aspartate is synthesised from aspartate and carbamyl phosphate via a proposed transition state shown in fig.2.



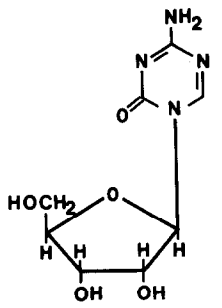
(XXIX)



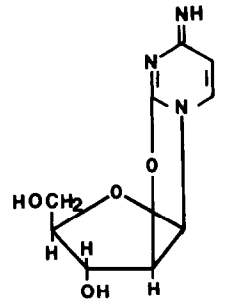
(XXX)



(XXXI)



(XXXII)



(XXXIII)

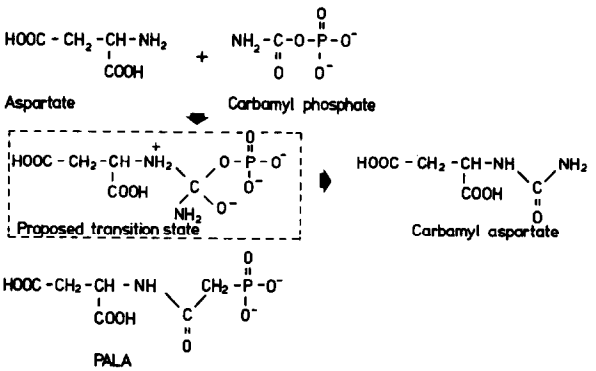



Fig.2. Design of transition state analogues. The antimetabolite (PALA) resembles in chemical structure the proposed transition state occurring during the enzymatic condensation of aspartate and carbamyl phosphate to form carbamyl aspartate (after Swyryd et al. [27]).

*N*-Phosphonoacetyl-L-aspartate (PALA) is an analogue of this proposed transition state and it has been shown to be a good inhibitor of pyrimidine biosynthesis. pyrimidine biosynthesis.

## 6. Spindle poisons and enzymes

A number of chemicals that have the ability to arrest cells in metaphase apparently do so by condensing with the microtubular protein vital for the formation of the spindle apparatus. As a result cells are arrested in metaphase [28]. Only two compounds, vinblastine and vincristine, are selective enough to be used in cancer chemotherapy. Despite their structural similarity, they are different in many of their biological properties, which is probably accounted for by cells being differentially permeable to the two chemicals.

Asparaginase is remarkably effective against animal tumours which do not have the asparagine synthetase enzyme and which rely on extracellular fluid as their sole source of asparagine. Unfortunately, not many human tumours are lacking the synthetase enzyme and those that do often quickly acquire resistance, with the result that asparaginase is used only in the treatment of acute leukaemia. Attempts to find other enzymes with anti-tumour activity in vivo have not been successful. Some, like glutaminase, can kill tumour cells in vitro, but glutaminase, unlike asparaginase, is very toxic to whole animals. An interesting finding has been that although the growth of both normal and tumour cells in vitro is inhibited by lack of methionine, normal cells can utilise tetrahydrofolate and homocystine in place of methionine, but tumour cells cannot do so. This suggests that some or all cancers may have a greater dependence on preformed methionine than normal tissues and suggests that a methioninase may be a selective anti-tumour agent [29].

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