# Some Biological Effects of Platinum Compounds

## NEW AGENTS FOR THE CONTROL OF TUMOURS

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***Certain complexes of the platinum group metals exhibit interesting biological effects. At low concentrations some are effective bacteriocides; others stop cell division and force bacteria to grow into long filaments. These complexes can also induce destruction of lysogenic bacteria. Most important, perhaps, some of these complexes are very potent anti-tumour agents against a broad spectrum of tumours, and may shortly be used for cancer chemotherapy in humans.***

In a truly heroic effort the National Cancer Institute of the U.S. Government has screened approximately 140,000 compounds for anti-cancer activity over the past 15 years. Of this number, however, only a dozen or so were inorganic compounds. This imbalance between organic and inorganic compounds reflects a fashion in chemotherapy that started over 35 years ago. Prior to that, heavy metals were extensively used in medical therapy. About 1935, the discovery of the sulfonamides introduced a new class of organic chemicals which were effective against many bacterial infections. Hard on this came the development of the antibiotics which coupled a high degree of efficiency with a low toxicity. At present, excepting a few outstanding drugs, heavy metals and other inorganic chemicals have ceased to be of active interest as chemotherapeutic agents. The screening program of the National Cancer Institute, occurring within the last 15 years, reflects this bias.

Recently a new class of potent anti-tumour agents has been discovered. These are inorganic complexes of the transition metal Group VIIIb. In this article we shall review briefly the history of this new development and discuss some of the properties that are now known concerning the activities of these compounds. It will be taken as granted that the general reader of this journal will find, as did the author, that biological and medical terminology are "terra incognita". To offset this to some extent, a glossary of the more important terms is appended to the article.

The germinal discovery was made about 1964 when platinum electrodes were used to apply an alternating electric field across a chamber in which bacteria were growing (1). Application of the electric field caused a cessation of all division in the E. coli rods, and since growth was not inhibited, resulted in the appearance of long filaments. A comparison of the normal appearing E. coli rods and the filamentous rods caused by the application of the electric field are shown under the same magnification in Fig. 1. Extensive detective work was required before we could conclude that the effective agent in blocking cell division in the bacteria was a small concentration ($\sim$10 ppm) of some platinum complex in solution, electrolytically formed by the applied electric field. It required much further work to determine that these complexes were *cis*-dichlorodiammineplatinum(II) and *cis*-tetrachlorodiammineplatinum(IV) (2,3).

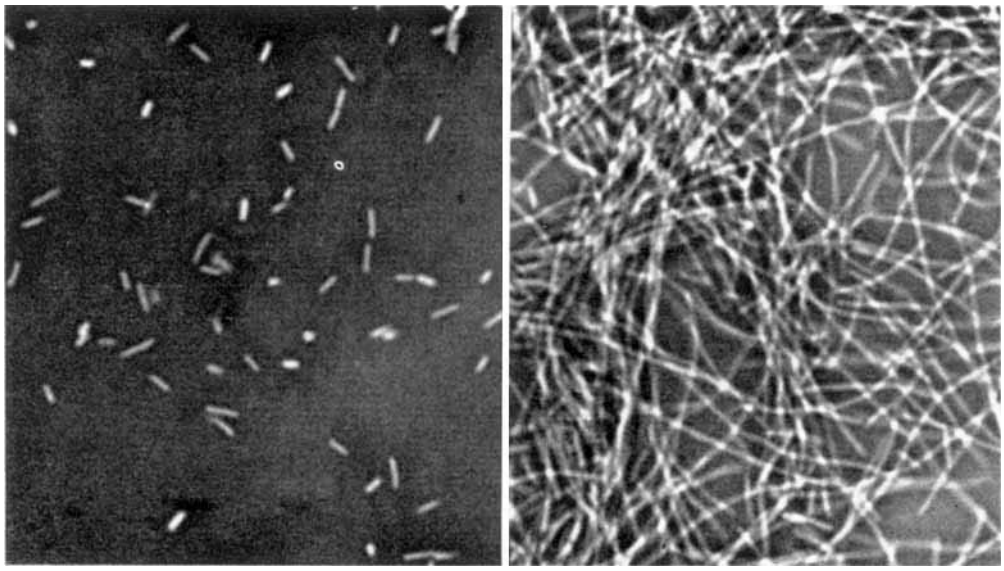


*Fig. 1 Phase contrast photomicrographs of E. coli B. × 600. (a) Normal bacteria grown in chemically defined medium. (b) Filamentous bacteria grown in same medium but incorporating 10 ppm of cis-dichlorodiammineplatinum(II). The ability of the cells to divide and separate is completely inhibited, so continued growth leads to long filaments*

The structures of these complexes and some other differently acting agents are shown in Figs 2, 3 and 4. With the verification that these platinum complexes were indeed capable of selectively blocking cell division in bacteria, a series of investigations was undertaken to determine the mechanism and the generality of the effect. VanCamp discovered that the hexachloroplatinum(IV) and tetrachloroplatinum(II) double negative ions are bacteriocides at low concentrations (1 to 6 ppm in the bacterial growth medium). These compounds undergo photochemical changes in the bacterial media, leading to a subsequent replacement of first one and then a second chloride by ammonia. The resulting neutral molecules are not bacteriocides until very high concentrations (~100 ppm in

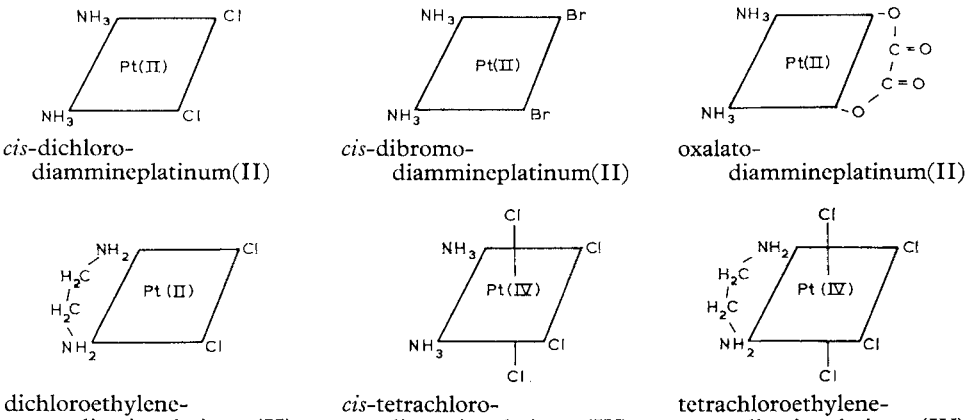
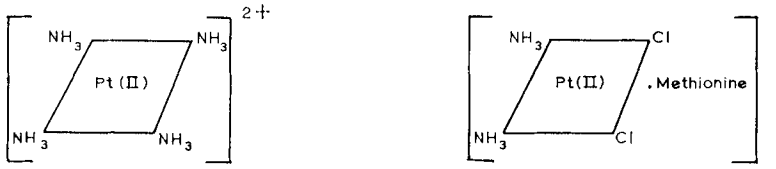



*Fig. 2 Structural formulae of active anti-tumour complexes of platinum. Only a very few specific configurations are active*




tetraammineplatinum(II) ion

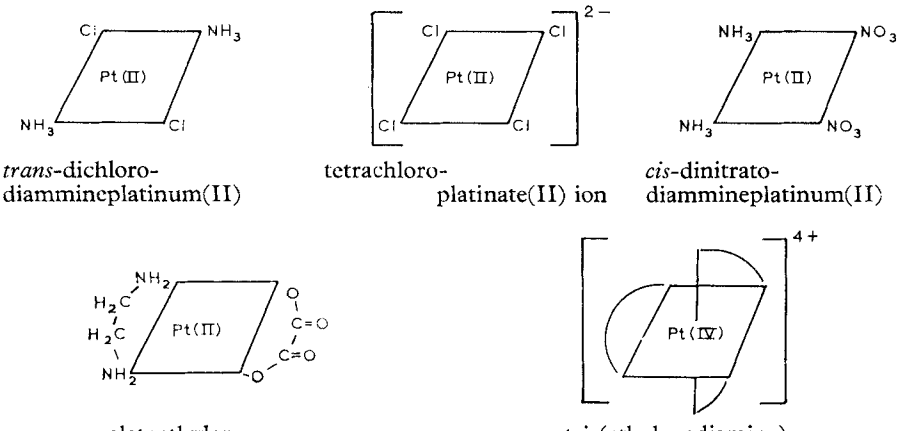
all complexes of *cis*-dichlorodiammine platinum(II) with methionine

*Fig. 3 Structural formulae of low toxicity, non-active complexes of platinum. Some soluble salts of the metals are tolerated very well in the animals, indicating that the activity is not due to a general heavy metal poisoning*

solution) are reached. Interestingly, the *trans* forms of these neutral molecules are not effective in blocking cell division in bacteria, while the *cis* isomers are. Thus we have two groups of compounds with different bacterial effects; the negative ionic species which are generally bacteriocidal, and the neutral species, in the *cis* form, which block cell division, but not growth. Gillard and his co-workers have reported similar effects in organic rhodium complexes (4), and Gale, Howle and Smith (5) have extended these observations to include photochemical reactions in ammonium hexachloroiridate, which also parallel the platinum transformations and bacterial activities.

A third class of bacterial effects of platinum compounds was discovered much later by Reslova (6). It is known that some strains of E. coli have previously been infected by viruses (bacteriophages) and that the genetic material of the virus was incorporated into and became part of the genetic material of the bacterial cell. The viral genetic material (genome) is repressed in these cells and is not normally detectable. Such bacterial strains are called "lysogenic" for the simple reason that a number of agents, such as ultraviolet light, X-rays, and some chemicals such as the nitrogen mustards (alkylating agents), are capable of inducing the development of partial or complete viruses which lead finally to the destruction (lysis) of the cell. The platinum compounds which are effective anti-tumour agents were found to be extremely potent in inducing lysis of such lysogenic bacteria.

Fig. 5 exhibits the three types of effects on the growth curves in test tubes of various




*trans*-dichloro-diammineplatinum(II)

tetrachloro-platinate(II) ion

*cis*-dinitrato-diammineplatinum(II)

oxalatoethylene-diamineplatinum(II)

tris(ethylenediamine)-platinum(II) ion

*Fig. 4 Structural formulae of high toxicity, non-active complexes of platinum*

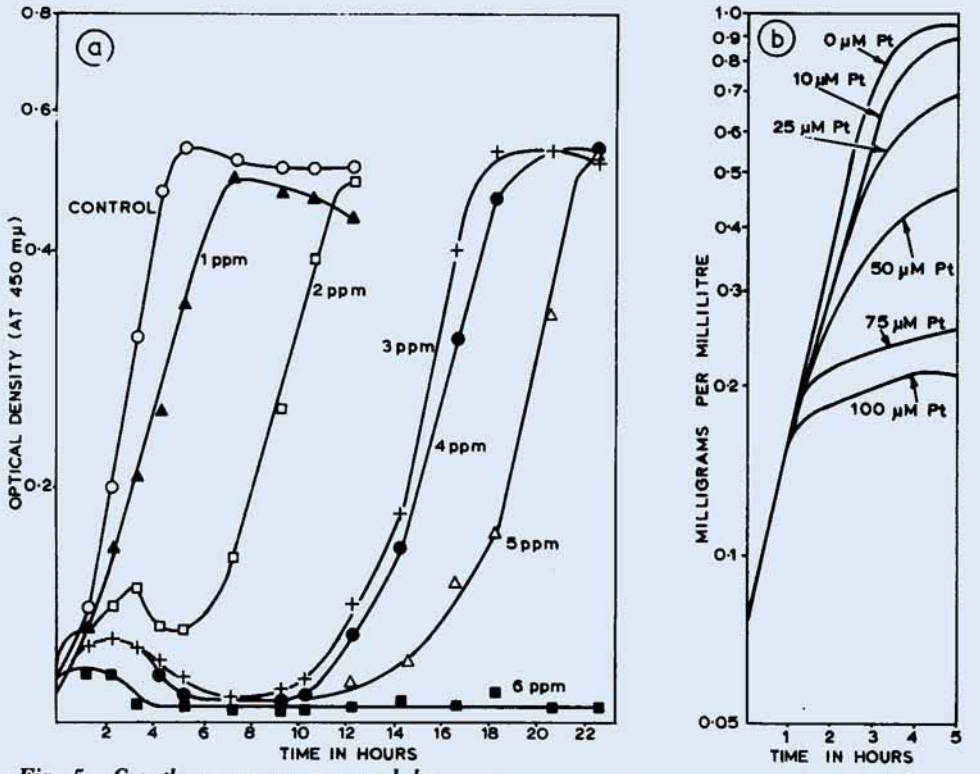



*Fig. 5 Growth curves, as measured by turbidity, of E. coli bacteria in test tubes in a chemically defined medium*

*(a) Bacteriocidal effects of $(NH_3)_2Pt(IV)Cl_6$ in concentrations ranging from 1 to 6 ppm. There is an increasing lag phase due to increasing percentage of cell kill (complete at 6 ppm), but rate of division (slope of lines) is not changed*

*(b) Filamentous growth curves in the presence of concentrations of cis-dichlorodiammineplatinum(II) ranging from 10 to 100 μM (2 to 20 ppm). After 1 hour, all bacteria are in the form of filaments. Only the growth rate is slowed down at higher concentrations*

*(c) A lysogenic strain of E. coli grown in the presence of a 10 ppm solution of cis-dichlorodiammineplatinum(II). After 3 hours the induced viruses cause complete lysis of the bacteria*

strains of E. coli reacting to the presence of different compounds of platinum, showing in Fig. 5a the bacteriocidal effect, in Fig. 5b the cell division inhibition, and in Fig. 5c the lytic effects of these compounds.

## Anti-tumour Activity

Since the platinum complexes of Fig. 2 are active in inhibiting cell division in bacteria, it suggested that they may also be interesting compounds to test as anti-tumour agents. Such a study was undertaken in our laboratory. We first determined the level of the platinum compounds which would be tolerated by the animals ($LD_{50}$ doses), and then whether, below these levels, they would inhibit the growth of transplantable tumours in these animals. The first test was with a

Sarcoma 180 tumour (a standard test tumour in cancer research) in Swiss white mice. We found that the animals would tolerate single injections of *cis*-dichlorodiammineplatinum(II) at a level (8 mg/kg) which could almost completely inhibit the growth of the transplanted tumour, while the $LD_{50}$ level was 14 mg/kg. This was the first evidence that these complexes are active anti-tumour agents (7). This compound was then submitted, along with *cis*-tetrachlorodiammineplatinum(IV) to the National Cancer Institute for screening against the Leukaemia L1210 tumour in mice, their present standard screening tumour. Their results showed that *cis*-dichlorodiammineplatinum(II) has a potent activity against this tumour, producing an increase in life span of the tumoured animals of 380 per cent, and a "cure" rate of 4 out of 10, with single injections at the therapeutic dose, 8 mg/kg.

In addition to inhibiting the development of newly transplanted Sarcoma 180 tumours in mice, it was later shown that one could wait for a period of 8 days for the transplanted tumour to grow to a very large size, before instituting treatment with the *cis*-dichlorodiammineplatinum(II). A single injection, intraperitoneally, of 8 mg/kg of this drug caused the complete regression of the large tumours in close to 100 per cent of the animals (8). These results are illustrated in Fig. 6. In addition to producing such extensive "cures" of advanced sarcomas, we found that even up to 11 months afterward, the animals remained immune to a re-challenge with the same tumour. Thus the "cure" produced long-lasting immunity to this tumour system (9).

This first anti-tumour complex of platinum has now been tested extensively on many other transplantable tumour systems. Kociba (10) demonstrated that it can produce 100 per cent "cure" in rats of the Walker 256 carcinosarcoma and the Dunning Ascitic Leukemia. Again, in his studies he has shown that one could wait until the tumour is in a highly advanced state, i.e., four days before the animal would die, before instituting treatment, and still rescue 100 per cent of the animals. The drug has been tested now at the National Cancer Institute and at the Chester Beatty Institute in London by Haddow and Connors (11) on a number of other tumour systems such as the Lewis Lung carcinoma, the B-16 melanocarcinoma, the P388 leukemia, and the ADJ-PC6A tumours. It has shown marked effectiveness in all of these tumour systems. The latter group have also shown a lack of activity of the complexes against the Rabbit VX2 carcinoma, the Gardner tumour, and a strain of the Walker 256 carcinosarcoma that is resistant to alkylating agents. At this time, a large number of other laboratories are investigating the anti-tumour activity of these complexes against a host of additional tumour systems. Their results will be reported in the near future. We conclude from this series of studies on the anti-tumour activity of *cis*-dichlorodiammineplatinum(II) that it is a very effective agent against a wide spectrum of transplantable tumours in animals. Anti-tumour activity has also been exhibited by the other compounds shown in Fig. 2. In some tumour types, other compounds have been shown to be more effective than the *cis*-dichlorodiammineplatinum(II). We have then a new class of drugs which could form a set of specific chemotherapeutic agents against specific types of tumours.

While transplantable tumours form the best screening systems for evaluating the effectiveness of new anti-tumour agents, there are other types of tumour systems which are more relevant to the cancers that occur in human beings. Two other such types of tumours are those caused by injections of chemical agents (carcinogens) into the animal which induce the formation of tumours, and those which are caused by the injections of certain classes of viruses. It is of interest, therefore, to determine whether these platinum complexes are capable of exhibiting anti-tumour activity against carcinogenically or virally induced tumour systems. Recently, Welsch (12) has investigated the activity of

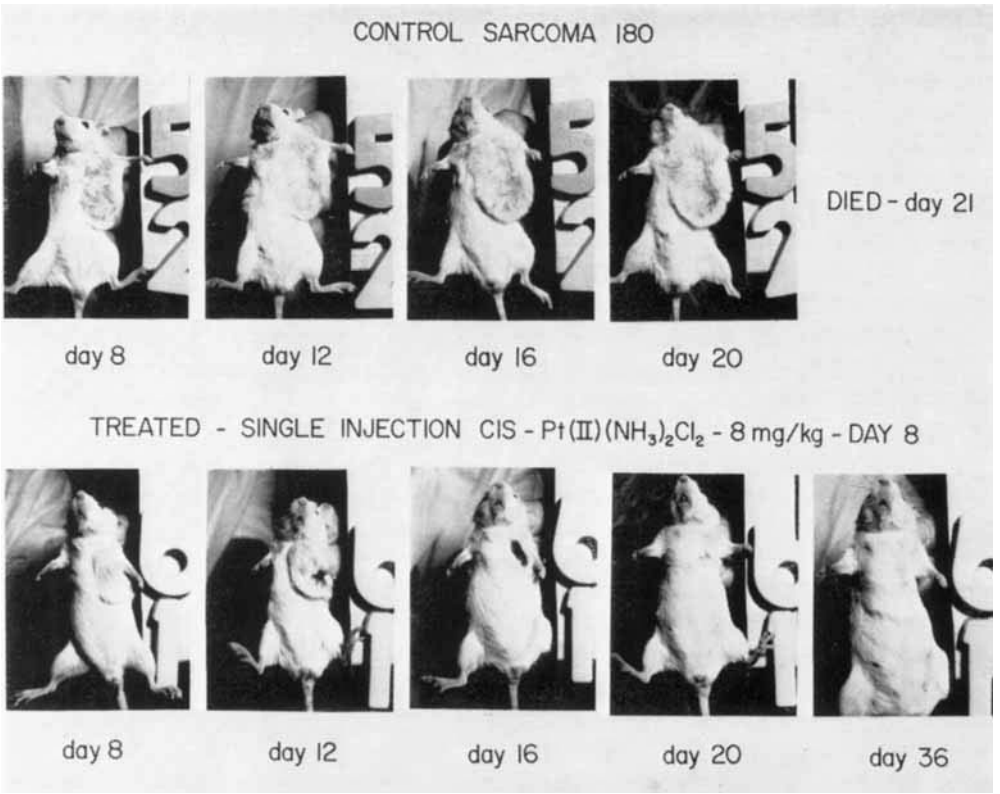



*Fig. 6 The regression of a large Sarcoma-180 tumour in the Swiss white mouse by a single intraperitoneal injection of* cis*-dichlorodiammineplatinum(II) at the therapeutic dose (8 mg/kg)*

*cis*-dichlorodiammineplatinum(II) against the dimethylbenzanthracene-induced mammary tumour in the rat. He has reported the complete regression of a very large number of extensively developed mammary tumours in the rats, with at least three out of 14 animals completely "cured" of all tumours. Since this particular tumour is the best model system for human mammary tumours, these results are of significant interest. Hinz (13) has tested the same compound against a virally induced tumour of the chicken. The chicks were injected with the Rous Sarcoma Virus, which, after a period of weeks, produces a large Sarcoma tumour in the wing web of the chick. Again, therapeutic doses of the complex were capable of causing complete regression of these tumours in 95 per cent of the animals. From the accumulating results of the anti-tumour activity of some platinum compounds against transplantable, carcinogenically induced, and virally induced tumours we can conclude that these compounds have one of the broadest spectra of action of any class of anti-tumour agents yet discovered. From the fact that it can cause regression of large tumours, and rescue animals when injected a few days prior to their death, we conclude that these compounds are very potent anti-tumour agents.

A major approach of the National Cancer Institute chemotherapy program is the use of combinational drug therapy. The basic idea is to mix drugs, all of which hit the target tumour, but each of which have different side effects. Usually these combination drugs have shown enhanced activity over and above the best of the individual drugs involved. However, only very few

combinations have been found which produced synergism of anti-tumour activity. Recently Venditti (14) showed that a combination of cytoxan (an alkylating agent) and *cis*-dichlorodiammineplatinum(II) produced a large number of "cures" against an advanced leukaemia L1210 in mice, whereas each of them individually produced only a small percentage increase in the lifespan, even up to toxic doses. This would appear to be a very promising method of treatment, since the drugs are both used at levels where side effects are minimal. I will describe below, in the section discussing the mechanism of action of the drug, a possible rationalisation for the apparent therapeutic synergism of these two drugs.

## The Toxicological Effects of Platinum Complexes

Experience teaches that it would be overly optimistic to expect an anti-tumour drug to be so specific in its action that it does not at all affect adversely the normal tissues of the body. In general, one finds that all such drugs have side effects which limit the dose levels that can be used in man. It is essential to predict from animal studies the kinds of toxicological effects one may anticipate when the platinum complex is used in human patients. Such studies have been undertaken in a number of laboratories.

It is first necessary to determine how long the drug remains in the animal after a pulse injection, and also its distribution as a function of time in the various organs of the animal. Toth-Allen (15) has evaluated the distribution in mice using a neutron activation technique which is sufficiently sensitive (~0.01 ppm) to measure accurately the low concentration of the platinum complex generally found in tissues (~1 to 5 micrograms per gram of tissue) after injection. She has reported that there is no specific uptake of the platinum complex in the Sarcoma 180 tumour tissue, and that indeed it appears in much higher concentrations in the filtering and excretory organs such as the liver and kidney. However, the damage to these latter organs is negligible, whereas the tumour, of course, is destroyed. A significant fraction (~15 per cent) of the injected drug is still detectable after six days. It has been found, both by Kociba and Toth-Allen, that the drug primarily affects those cells of the body which are most rapidly dividing, such as the cells of the intestinal lining, and of the bone marrow. It is, therefore, a cytotoxic drug, and must be used with caution. These side effects are those generally expected with anti-tumour drugs and techniques for their amelioration have been developed. In the case of the platinum complexes, the damage is always reversible, and the animals recover. They exhibit no long-lasting side effects caused by therapeutic treatment.

## The Molecular Biology of Platinum Complexes

The interaction of the platinum complexes with cellular macromolecular syntheses has been investigated by Harder using tissue culture techniques (16). He has measured the ability of treated cells to manufacture nucleic acids (DNA and RNA) and proteins, using radioactively labelled precursors for each of these species. Only those platinum complexes which are active anti-tumour agents are capable of inhibiting the synthesis of DNA. Those which are inactive do not produce such inhibition. Further, at the dose level that appears in the tumour tissues of animals, the DNA replication is selectively inhibited, while RNA and protein synthesis are not inhibited. Similar results have been found by Howle and Gale *in vivo* (17). Such results are shown in Fig. 7. Harder has further proved that the inhibition of DNA synthesis is not caused by a blockage in the synthesis of the necessary precursors of DNA, nor is the ability of these precursors to enter the cells impaired. We conclude from these data that the platinum complexes most probably act by causing a primary lesion in the DNA of the cell. It is of interest now to determine the nature of this primary lesion.

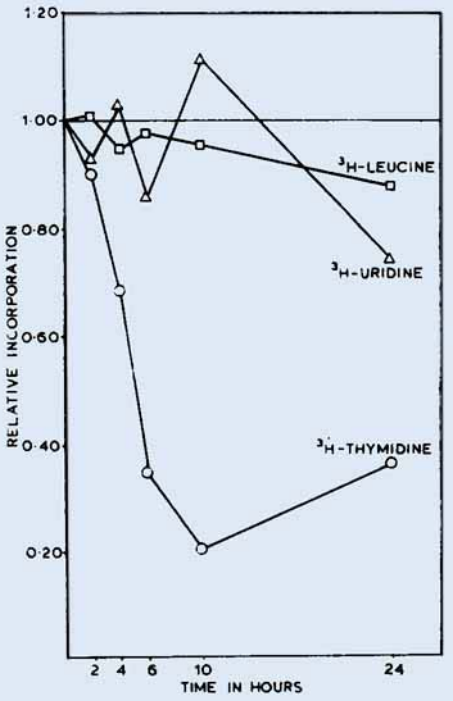



***Fig. 7 The selective inhibition of DNA synthesis in human $AV_3$ cells grown in tissue culture by exposure to 5 μM (1 ppm) cis-dichlorodiammineplatinum(II) as measured by the rate of uptake of tritiated thymidine. The RNA synthesis, measured by the rate of uptake of tritiated uridine, and protein synthesis, measured by the rate of uptake of tritiated leucine are close to the control values (the horizontal line at 1.00 per cent)***

Drobnik (18) suggested, on the basis of the similar occurrence of the two active chloride groups in bifunctional alkylating agents and in the *cis*-dichlorodiammineplatinum(II), that the platinum complexes act similarly to the bifunctional alkylating agents. Information we have gathered up to now indicates that there is indeed a similarity in the actions of both of these classes of compounds, but it is by no means an identity. For example, they are both capable of forcing giant cell formation in mammalian tissue culture, and of forcing filamentous forms in bacteria. They both selectively inhibit DNA synthesis at low concentrations in mammalian cells, and are very effective in inducing lysogenic strains of bacteria. The present understanding of the action of the bifunctional alkylating agents is believed to be the formation of an interstrand crosslink between the guanine bases, at the N-7 position, in double stranded DNA. In order to accomplish this interstrand crosslink, the two active chloride groups must be approximately 8Å apart. In the *cis*-dichlorodiammineplatinum(II), the spacing between the two active chloride groups is 3.3Å. It would appear, therefore, that such platinum complexes cannot be causing the same primary lesions, i.e., interstrand crosslinking, as do the bifunctional alkylating agents. On the basis of some recent evidence that the platinum complexes inhibit a single stranded bacteriophage as well as it does the double stranded bacteriophage (19); that it reacts *in vitro* primarily with purine bases rather than pyrimidine bases; and taking into account that the stacking spacing of the bases in the Watson-Crick model of DNA is about 3.4Å, we are led to suggest that the primary lesion caused by the platinum complexes is an *intrastrand purine dimer*. This is a new type of lesion that has not been investigated before. *In vitro* studies with purine dimers tend at this time to validate this hypothesis, but much further work must be done to prove or disprove it.

If indeed the platinum complexes do form an intrastrand purine dimer, while the bifunctional alkylating agents form an interstrand crosslink, it may then be that a cellular repair mechanism, which normally operates within the cell to eliminate lesions in the DNA, would have more difficulty in simultaneously repairing both types of lesions, than either one of them individually. This could provide a rationale for the therapeutic synergism described above.

## Speculation on the Mode of Action of the Anti-tumour Effects

Reslova (6) has shown that all platinum complexes which are active anti-tumour agents are capable of inducing lysis in lyso-

genic bacteria; those which are non-active do not produce such lysis. These results provide a clue as to the mode of action of the platinum complexes in specifically damaging tumour cells. It has been suggested by a number of scientists that viruses may be the sole agents which transform normal cells into tumour cells. However, since viral particles (virions) are rarely found in tumour tissues, it is more likely that the mechanism of transformation operates after viral infection by the incorporation of the viral genome into the cellular genome. Normally this viral genome is repressed and unable to exhibit its presence. Agents such as X-rays, radiation, other viruses, and chemical carcinogens could partially derepress these viral genomes, forcing the manufacture of a number of new proteins in the cell. It is hypothesised that some of these new proteins are the active agents causing transformation into tumour cells. This hypothesis is consistent with the theory of Huebner (20). If we now take the evidence of Reslova, obtained from bacterial cells, and apply it to the mammalian cells, we can suggest that the primary lesions in the DNA caused by the platinum complexes act to derepress completely the viral genome. This then leads to the active multiplication of the viral particles, which may or may not be defective. In any case this will almost certainly increase the antigenicity of the cell, and therefore stimulate the immune system to produce retaliatory antibodies to the tumour cells at an enhanced rate. It is also possible that, if the viral genome and the viral particles can be eliminated from the cell without concommitant cell death, the cell should revert back to its normal status. Such effects have been known to occur in the past. This speculation suggests a number of possible tests, and these are under way in a number of laboratories.

## Future Outlook

One platinum complex, the *cis*-dichlorodiammineplatinum(II) is currently on test at the National Cancer Institute in preclinical trials. It is anticipated that it will be tested on human patients some time in the spring of 1971. It will be many months before sufficient clinical data will be available to allow a judgment of the efficacy of this new compound, either alone or in combinational drug therapy, in human tumours.

In the meantime, research must be pursued to develop analogues of the original compounds, and to continue testing these analogues on screening tumour systems. Without hesitancy I suggest it is now appropriate for inorganic chemists to join their organic brothers in submitting samples of their syntheses to appropriate Cancer Institutes for screening for anti-tumour activities. In addition to this chemical synthesis activity, it is desirable to know in more detail, and with greater security, the mechanisms of action of this new class of drugs, as well as their general toxicological and pharmacological properties in mammalian systems.

This class of complexes of the platinum group metals form a promising new class of drugs to be added to the medical armamentarium.

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## GLOSSARY

**Alkylating agent.** A reactive chemical which replaces a hydroxylic hydrogen atom by an alkyl group. Bifunctional alkylating agents have two attachment sites (e.g., nitrogen mustard); monofunctional alkylating agents have one available attachment site (e.g., methylmethanesulphonate)

**Antibodies.** Protein components (gamma globulins) of an immune organism which attach, with great specificity, to antigens and incapacitate them.

**Antigen (antigenicity).** Any substance (usually proteinaceous) which when injected into an organism causes the formation of antibodies.

**Bacteriocide.** Chemical agents which kill bacteria, usually important only if they produce tolerable effects in the host organism.

**Bacteriophages.** A class of viruses which attack bacteria only.

**Carcinogen.** Any agent (chemical, physical, bacterial or viral) which can produce a cancer in an organism.

**Carcinoma.** A specific form of cancer which is a malignant tumour of lining cells and glands in the body of an organism.

**Carcinosarcoma.** A mixed tumour with characteristics of both a carcinoma and sarcoma (see below).

**"Cure".** A complete disappearance of all detectable symptoms of a cancer for a minimal period of time (in humans, it is usually about five years). The double quote marks usually imply that the results are reported before this minimal time has elapsed.

**Cytotoxic.** Refers to cell destruction or damage, usually caused by agents deleterious to any essential process of the cell.

**Cytoxan.** Commercial name for cyclophosphamide, a very potent anti-tumour agent of the bifunctional alkylating agent class.

**DNA.** Deoxyribonucleic acid. A very long chain polymer consisting of alternating phosphate and sugar groups with attached purine and pyrimidine bases. The genetic information of the cell is coded in the sequence of these bases.

**Escherichia Coli (E. coli).** A bacterium found in normal intestines. It is probably the most intensively studied organism in biology.

**Genome.** The total genetic information of a cell.

**Interstrand Crosslinks.** A chemical (covalent) link between two bases, each situated on one of the two strands of double stranded nucleic acid (DNA, RNA). It is generally believed that this link prevents the separation of the two strands which is necessary for genetic replication.

**Intrastrand purine dimer.** A chemical bond (covalent) formed between two adjacent purine bases (adenine or guanine) attached to the same strand of a nucleic acid molecule.

***In Vitro.*** Refers generally to experiments done in a cell-free system. Here, however, it also encompasses the growth of mammalian cells in dishes.

***In Vivo.*** Refers to experiments done in an intact organism.

**$LD_{50}$.** The dose, given under specified conditions, of any agent, which causes death in 50 per cent of the organisms.

**Leukaemia.** A mass of relatively undifferentiated cells in uncontrolled growth, disseminated in the blood system. Specifically a disease of the blood and blood forming organs characterised by a permanent increase in the number of white blood cells.

**Lysis (Lytic).** The bursting of a cell caused by the destruction of the cell membrane.

**Lysogenic bacteria.** Bacteria that contain the genetic information (genome) of a bacteriophage incorporated in the cellular genome.

**Melanoma.** A tumour containing melanin, the black, polymeric pigment normally made in special skin cells.

**Purines.** Aromatic, nitrogen-containing, ring molecules (adenine and guanine) with basic properties. One of the two classes of bases defining the genetic code by their sequences in nucleic acids.

**Pyrimidines.** Cytosine and thymine. The second class of aromatic, nitrogen-containing ring structures, that occur in nucleic acids, and define the genetic code.

**Repression (derepression).** The forcing of a gene into an inactive state. A repressor molecule, believed to be a protein, blocks substances which turn genes on, or interferes with the genes' ability to eventually produce a specific protein.

**RNA.** Ribonucleic acid. A long chain polymer consisting of alternating phosphate and sugar groups with attached purine and pyrimidine bases. May be the sole source of genetic information in the organism, and additionally acts as an intermediary to transfer the genetic code into specific protein manufacture.

**Rous Sarcoma Virus.** An RNA containing virus which is the causative agent of tumours in fowl.

**Sarcoma (S-180).** A specific form of cancer which is a malignant tumour of supporting tissue in the body of an organism.

**Virus (Virion) Defective-Active.** Infectious disease-causing agents which are smaller than bacteria and which always require an intact host cell for replication. They may contain either DNA or RNA as the genetic material. The nucleic acids may be double stranded or single stranded. Defective viruses are incapable of infecting host cells, or cannot replicate once their genome has invaded the cell.