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TRANSITION METAL COMPLEXES IN CANCER CHEMOTHERAPY

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CONTENTS

ABBREVIATIONS

acac	acetylacetonate anion, $CH_3.CO.C^{-}H.CO.CH_3$
DMG	dimethylglyoxime
S.180	Sarcoma 180
mp	6-mercaptopurine
tgn	thioguanine
butp	butylthiopurine

en	ethylenediamine	Et	ethyl
dien	diethylenetriamine	*o*-phen	*o*-phenanthroline
py	pyridine	OAc	acetate
ox	oxalate	gly	glycine anion
mal	malonate	DMSO	dimethyl sulphoxide
Me	methyl	DMBA	dimethylbenzanthrene

A. INTRODUCTION

Cancer chemotherapy is a field of cancer treatment which has largely evolved in the last thirty years. The essential backbone of the subject has been the various screening processes employed as tests for drug activity. Outstanding among these has been the work of the U.S. National Cancer Institute (N.C.I.), which has tested around 150,000 compounds in the past two decades. Many compounds are tested as a result of specific biological observations but others, probably the majority, have been screened on a purely empirical basis. The intelligent application of the empirical approach has led to the discovery of useful "families" of anti-cancer drugs. Once random screening has unearthed a potential drug, systematic screening of compounds with related structures enables any structure activity relationships to be exploited in order to maximise the anti-tumour effect. Perhaps not surprisingly, prior to 1969 very few of the screened compounds were inorganic in nature (less than 20 out of 150,000 for the N.C.I.). However, in 1969 Rosenberg and Van Camp demonstrated potent anti-tumour activity for certain Pt coordination compounds[1,2] and this key discovery has opened up a new class of anti-tumour agents, namely inorganic coordination complexes. Work is now in progress in several laboratories to redress the balance of screening more towards inorganic compounds, and it is hoped that this review will encourage the trend.

Chemotherapeutic research depends heavily on the screening and testing procedures and it is important to have some knowledge of these in order to interpret the results, and to determine the emphasis to be put on them in the context of a systematic testing programme. Thus it is necessary to outline these procedures. Several reviews have successfully achieved this aim in more detail than is desirable here, and they are highly recommended to the reader[3-5]. An ideal anti-cancer drug will be specifically toxic for cancerous cells as opposed to normal cells. This ideal has proved to be elusive and a common property among all cancers is needed if a universal cure is to be found. Such a feature has, of course, yet to be discovered. Some chemicals are selectively toxic towards cancer cells of a particular type, but this is a property of cell type rather than a general property of cancer. The term cancer embraces many different diseases which differ from one another biochemically. This would not be quite such a huge problem if each human cancer had an animal counterpart which could be used for transplantation and testing. Unfortunately this is manifestly not the case.

The immune response to advanced cancers is believed to be very small and the cytotoxic agent must kill virtually every neoplastic cell if a recurrence is to be prevented. Drugs tend to kill a fixed percentage of cells (in animals) and single administrations rarely bring about complete eradication of a tumour[3]. Repeated administrations are necessary and these should be continued after the tumour has apparently disappeared. Thus the drug must be reasonably well tolerated.

(i) Screening tests

(a) In vitro

Most anti-tumour agents have proved to be selectively toxic for cells undergoing DNA synthesis and not surprisingly there is some correlation between anti-tumour effects and effects on microbial or other rapidly growing organisms. Rosenberg's discovery of the anti-cancer platinum compounds hinged on an observation of bacteriological activity[6,7] (see below). However, in general these systems are most useful for detailed mechanistic studies after initial detection of anti-tumour activity. Human or animal cells in culture are used as screens but these give only cell toxicity data and no information on the drug's overall effectiveness.

(b) In vivo

Ideally animals bearing spontaneous tumours should be used but these tend to arise late in the lifespan and are inconsistent from animal to animal. Thus in practice they are used only as a secondary or more specialised screen. Most spontaneous or chemically-induced tumours can be transplanted to other animals by tumour fragments, which grow immediately. This is the basis for almost all screens and such systems have been at least partly responsible for the discovery of all the anti-tumour drugs in use today (Table 1). A large number of tumour lines has been transplanted but there are a few which are most commonly used (Table 2). The National Cancer Institute has now set up protocols for 20 in vivo test tumour systems[8]. Mouse tumours are most popular for primary screens as relatively small quantities of drug are required, especially by comparison with rats. Mice of the same age and sex are used, generally with as much in-breeding as possible to give an approximately "homogeneous set" of animals. Particular tumours often appear to be more sensitive to a particular type of drug (Table 2). No particular screen seems to be sufficiently sensitive to all known drugs to be used as a universal primary screen (although Leukemia L.1210 is most widely used and would have detected the majority of the drugs in clinical use). In this situation it seems wise that a variety of screens should be employed even on the same family of compounds.

(ii) Screening parameters

Screening should determine whether any anti-tumour effect is present up to the

TABLE 1
Clinically useful anti-tumour agents

Drug type	Description	Examples	Mode of action
1. Antimetabolites	Related in structure to a metabolite and competes with it for an enzyme	(a) Antifolates: e.g. methotrexate	Inhibits synthesis of purines and thus DNA
		(b) Antipurines: e.g. 6-mercapto-purine	Inhibits synthesis of purine nucleosides and thus DNA
		(c) Antipyrimidines: e.g. 5-fluorouracil	Inhibits synthesis of pyrimidine nucleosides and thus DNA
2. Alkylating agents	Reactive alkyl group donors. React at many centres, particularly DNA	(a) Bifunctional: e.g. mustard gas	Inhibits DNA synthesis, probably by cross-linking opposite strands
		(b) Metabolised agents: e.g. cyclo-phosphamide	Converted to active alkylating agent in vivo (e.g. by liver). Acts as above
3. Natural products		(a) Antibiotics: e.g. Actinomycin D	Varied, often inhibits m–RNA formation
		(b) Alkaloids: e.g. Vincristine	May affect mitosis by reaction with –SH groups in the mitotic apparatus
4. Steroids and hormones	Varied. Effective against tissues whose growth is normally under hormone control	Oestrogens, progesterone	Cancer cells are still subject to some degree of hormone control

TABLE 2
Some commonly used transplantable tumours

Name	Species	Origin
Sarcoma 180 (also known as the Crocker)	Mouse (various strains)	Arose spontaneously in the axilla of a white mouse. Initially described as a carcinoma but after many transplantations it has the features of a sarcoma. Moderately sensitive to a number of agents and is used in a solid and ascitic form
Carcinoma 755	Mouse (C.57 Black)	Arose spontaneously as a mammary adenocarcinoma. Slow growing and sensitive to antimetabolites
Leukemia L.1210	Mouse (DBA and F_1 hybrids)	Chemically induced by cutaneous application of 20-methylcholanthrene. Grows from a single cell and disseminates rapidly in both solid and ascites forms. Very sensitive to many classes of agent, particularly antimetabolites
Ehrlich ascites	Mouse (various strains)	One of the earliest known transplanted tumours. Arose as a spontaneous mammary tumour in a stock mouse. Solid and ascitic forms used. Not very sensitive to known chemotherapeutic agents
$6C_3HED$ lymphosarcoma (also known as the Gardner)	Mouse (C3H and CBA strain)	Induced in a female C3H mouse by equilin-benzoate. Solid and ascites forms used. Sensitive to many classes of anti-cancer drug but especially asparaginase
Walker carcinoma	Rat (various strains)	Arose as a spontaneous mammary tumour in a stock rat. Rapidly growing tumour which quickly becomes necrotic. Solid and ascites used and both are particularly sensitive to alkylating agents
Yoshida sarcoma	Rat (various strains)	Arose in the scrotum of a rat fed with 2-amido-azotoluene. Solid and ascites forms used. Moderately sensitive to many agents, but particularly sensitive to alkylating agents

toxic dose, and should enable comparisons to be made with other compounds. Important parameters are defined as follows:

Lethal doses are represented by $L.D_n$, where n is the percentage of animals killed at that dose. $L.D_{50}$ is most commonly used.

Similarly *effective* or *inhibitory* doses are represented by $E.D_n$ or $I.D_n$ respectively. $I.D_{90}$'s are most commonly quoted.

The *therapeutic index* (T.I.) is the ratio of a lethal dose (usually $L.D_{50}$) to an inhibitory dose (usually $I.D_{90}$) and this indicates the selectivity of the drug.

For solid tumours the amount of inhibition is measured by a comparison of the

weights of treated and untreated (control) tumours. This is expressed as a percentage and termed *T/C*. Values less than 50 are generally considered significant.

For ascites and leukaemias the mean survival time is compared to that of the controls. Any percentage *increase in life span (I.L.S.)* is a measure of anti-tumour activity.

In mice the drugs are usually administered intraperitoneally dissolved or suspended in a suitable solvent, either as a single dose or on a daily basis. Four widely spaced tests can roughly determine the toxicity. Six closely spaced tests up to the toxic level should then enable the $L.D_{50}$ and $I.D_{90}$ to be calculated. Drugs are administered either the day after transplantation, when the tumour is just palpable, or sometimes when the tumour has reached an advanced state. At the end of a fixed time the test animals and controls are killed and the tumours are dissected out and weighed. Screens are routinely checked against compounds of known T.I. which act as a positive control against histological changes in the tumour line.

B. A SURVEY OF RESEARCH PRIOR TO THE DISCOVERY OF ANTI-TUMOUR PLATINUM COMPOUNDS (1969)

Relatively little systematic research in this area was attempted prior to 1969. Much of the interest was centred on the potential carcinogenicity of metals and their salts, rather than on any potential anti-cancer properties. There are several extensive reviews on metal carcinogenesis[9,10]. Another important area involved the study of relative concentrations of essential metals in neoplastic and normal tissue with the aim of finding differences which might be exploitable in terms of cancer control[11–15]. It is intended that this review should concentrate on transition metal complexes, although other inorganic compounds will be mentioned where pertinent.

The first metal-based remedy to undergo extensive clinical testing was Fowler's solution, which originally consisted of a suspension of lead arsenate in benzene[16]. Similarly colloidal lead and lead phosphate have been tested with some reported success[17]. The use of combinations like these leads one to presume that less emphasis was placed on general toxicological studies than is the practice today. However, arsenic, in the form of sodium or potassium arsenite or various organoarsenicals, is still used clinically to a limited extent and particularly in the treatment of leukaemias[18–20]. Elemental germanium has shown some activity against spontaneous mouse tumours but tin and vanadium were found to be inactive[21]. Small doses of copper, bismuth, ruthenium and selenium have also been administered clinically[16].

(i) General studies on various metals

One of the earliest extensive surveys[22] of the anti-tumour actions of inorganic compounds was made by Collier and Krause (1931). Sixty four assorted metal com-

TABLE 3
Effect of metal chlorides on a sarcoma (DBA 131) in DBA mice (ref. 23)

Compound	Drinking water			Daily injections (5 days)		
	Dose (mg/day)	*T/C*	Percentage change in body wt.	Dose (mg/day)	*T/C*	Percentage change in body wt.
$CoCl_2$	0.7	65	−8	0.1–0.2	73	−2
$CuCl_2$	0.3	68	−10	0.1–0.2	75	−2
$HgCl_2$	0.3	63	−9	0.05–0.75	85	+3
$NiCl_2$	0.4–1.0	81	+2	0.2	64	+3
$RhCl_3$	0.2	76	+7	0.1	100	+5
RbCl	0.4	76	−7	0.5	99	−1
$AgNO_3$				1.0	65	−4
$TlCl_3$				0.1	71	−3
$ZnCl_2$	0.4	59	−8	0.1	96	−2

(The compounds were often not tested up to toxic levels)

pounds including oxides, halides, cyanides and ammines (Cu, Pb, Cr, Mn, Fe, Co, Ni, Ru, Rh, Os) were dissolved or suspended in physiological saline or olive oil and were injected subcutaneously into mice bearing an implanted Ehrlich carcinoma. Unfortunately no quantitative data were given, but some salts of lead, chromium and manganese were described as slightly active while $Cs_2[RhCl_6] \cdot H_2O$ in oil was considered distinctly active. $K_4[Ru(CN)_6]$ and OsO_2 in oil also registered marginal effects. Taylor and Carmichael (1953) assessed 37 metal chlorides and nitrates, noting their effects on the embryo and tumour of tumour-bearing eggs[23] (the implanted tumour was a mouse mammary adenocarcinoma) and also on DBA mice bearing a sarcoma transplant. The compounds were generally ineffective (Table 3), showing a maximum of 41% inhibition against the sarcoma, and this was when administered in the drinking water. Platinum chloride, $PtCl_4$, showed little effect (< 20%) and proved to be very toxic towards the embryos. Geschickter and Reid (1947) synthesised oil-soluble phthalate complexes[24] (Fig. 1) of cobalt, nickel, iron, copper and manganese, all in the bivalent state. No preparative or analytical details are given for these compounds and their actual stoichiometry must be in doubt. However, the copper,

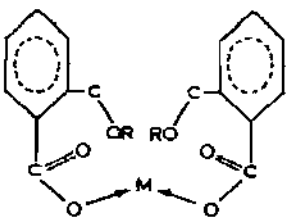


Fig. 1. Oil-soluble phthalate complexes (R = alkyl group, most commonly butyl).

nickel and cobalt buthylphthalate complexes were tested on humans. Twenty cases of leukaemia were treated and it was claimed that seven benefited from this treatment. Although the compounds appear to have had some selective toxicity towards the leukaemic phase of the white cell count, the response to therapy, when favourable, produced a regression which lasted only 3–6 weeks. Following this it was necessary to employ 2–5 times the original dose to secure a second regression. The nickel compounds showed an effect against spontaneous lymphomas in D.B.A. mice and oestrogen-induced mammary carcinomas in rats, giving a considerable increase in lifespan in each case, although the tumour remained static rather than decreasing in size. Subsequently nickel butylphthalate showed useful clinical effects on some solid human tumours. This apparently promising research appears to have been discontinued; in the light of recent work (particularly on carboxylates, p. 371) it would seem worthy of re-investigation including a complete characterisation of the complexes.

Balo and Bangu (1957), working on the theory that the oxidative metabolism in tumour cells is suppressed, looked for autoxidisable complexes which might promote the oxidation mechanism and hopefully inhibit the tumour growth[25]. They claimed to have prepared some fifty different complexes of the metals found in enzymes, with ascorbic acid and the metabolites of the Krebs cycle (carboxylic acids) as ligands. However, results for only three such complexes are detailed. These are iron and cadmium ascorbate and manganese malate complexes, which were tested against an Ehrlich mouse carcinoma and a Guerin rat carcinoma. The iron complex enhanced the growth of the mouse tumour, while the cadmium one, at identical doses, showed an inhibitory effect but was very toxic. The manganese malate was active against both tumours and was less toxic. The iron ascorbate inhibited the oxidation of *p*-phenylenediamine which had been added to the carcinoma tissue, whereas the cadmium complex enhanced the corresponding oxidation. The manganese malate formed a coloured complex with *p*-phenylenediamine which decolourised quickly in normal organs but only slowly in tumour tissue. Although these tests appear to support the original hypothesis, they obviously constitute insufficient evidence for it to be considered proven. However, this approach seems worthy of further investigation.

Ru^{II} and Cu^{II} complexes with 3,4,7,8-tetramethyl-1,10-phenanthroline (phen) were evaluated against the Landschutz ascites tumour in mice[26]. $[Cu(phen)_2]Cl_2$ and $[Ru(phen)_3]Cl_2$ caused significant inhibition while $[Ru(phen)_2(acac)]Cl$ was less effective[27]. Similar tris-chelates have shown neuromuscular toxicity (p. 380).

(a) Copper

Simple copper salts appear to have some cytostatic properties, and $CuSO_4$ has produced significant inhibition of the tumour growth rate of S.180 and various experimental carcinomas (including Ehrlich)[28]. However, no significant prolongation of survival time was recorded. Various oximes were tested against the Ehrlich ascites and Sarcoma 180 tumours with little effective increase in lifespans (I.L.S. $< 30\%$)[29,30].

In vitro studies indicated that some of these compounds had an injurious effect on the selective permeability of the tumour cell membranes, and this effect appeared to be increased in the presence of Cu^{II} and Fe^{II} salts. For copper and iron α-ketoglutarate complexes the effect was apparent at 25 mg/ml as opposed to 100 mg/ml for the free ligand. Dimethylglyoxime, DMG (< 50 mg/kg/day), showed no inhibition of the test tumours, but gave good results in the presence of Cu^{II} although not with Ni^{II}, Co^{II}, Zn^{II}, Mn^{II}, Mg^{II} or Hg^{II}. The $[Cu(DMG)_2]$ chelate gave an increased lifespan of 200–300% and showed activity even on a single injection of 5 mg/kg. These compounds were initially tested on the basis that the metal might mask the hydrophilic group of the oxime and increase its permeability through the cell membrane. Once in the cell, the anti-tumour activity of the free oxime (which appears to be quite small in vivo) might be displayed. A similar approach was adopted later by Kirschner using antimetabolite drugs as ligands[31,32]. Anti-tumour activity has also been recorded for copper chelated with thiosemicarbazones. The most investigated system involves 3-ethoxy-2-oxobutyraldehyde bis-thiosemicarbazone (known as kethoxal bis-thiosemicarbazone, KTS) (Fig. 2), the activity and toxicity of which was found to be directly proportional to the dietary intake of Cu^{II} for rats bearing the Walker 256 carcinosarcoma. The copper alone had no effect while Co^{II} ions reduced the activity and toxicity of both KTS and the Cu–KTS combination. The Cu–KTS chelate was synthesised and gave dose-dependent tumour regression of the Walker 256 in rats, although the results do not appear to be significantly better than those for KTS alone[33–35]. Other workers have reported activity for the Cu–KTS chelate against S.180 in mice while chloride and stearate salts of Cu^{II} have no effect[36,37]. Cu^{II} ions had a similar effect on pyruvaldehyde bis-thiosemicarbazone against a variety of tumours while Zn^{II} also enhanced the activity against the Walker 256 in rats[38]. Less effective were Mn^{II}, Mg^{II}, Fe^{II}, Ni^{II} and Cd^{II} in decreasing order. A consistent inhibition of S.180 in mice has been reported using copper, palladium and cadmium complexes with dithioxamide (rubeanic acid)[39]. The tumour diameter is quoted as $\frac{1}{2}$–$\frac{1}{3}$ of the controls. The Cu compound was inactive against a leukaemia (L4946 in AKR mice) but the lead analogue gave some increase in lifespan. The latter also caused some inhibition of a melanoma (Harding–Pusy), but was ineffective against a carcinoma (RC in DBA mice). Dithioxamide was inactive in all cases.

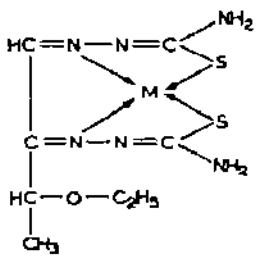


Fig. 2. Chelate complexes of 3-ethoxy-2-oxobutyraldehyde bis(thiosemicarbazone), KTS.

(b) Cobalt and chromium

Japanese researchers have studied a series of Co^{III} and Cr^{III} ammine complexes which were evaluated against the Yoshida sarcoma[40,41]. Some forty compounds were examined but none showed better than marginal activity. The Co^{III} hexammine and pentammine salts were very toxic (confirming the earlier work of Dwyer et al.[105] on inert complexes with positive charges — see p. 380) but $[Co(NH_3)_4Cl_2]Cl$, $[Co(NH_3)_4CO_3]NO_3 . \frac{1}{2}H_2O$ and $[Cr(NH_3)_4(C_2O_4)]$ $[Cr(C_2O_4)_2(NH_3)_2]$ showed some inhibition of tumour growth. It is interesting to compare the configuration of these complexes (all *cis* except the chloride, which is not identified as *cis* or *trans*) with those of platinum which are described in the next section. It is reported that a 1 : 1 complex between bis-histidinecobalt(II) and 8-azaguanine is somewhat effective against Ehrlich ascites in mice. It was excreted unchanged in the urine and it is claimed that the oxidation of succinic acid by liver cells was normal while in the cancer cells it was depressed. The complex had a specific effect on cytochrome oxidases[42]. Similarly a cobalt complex with cysteine and phenylthiourea prolonged the life of mice with an Ehrlich carcinoma[43]. Cobaltous chloride, $CoCl_2$ and $Na_2[Co(NO_2)_6]$ did not affect the growth of established epithelial tumours induced by methylcholanthrene, although both were inhibitory when given during methylcholanthrene administration[44]. Several naturally occurring cobalt-containing molecules such as cyanocobalamin (vitamin B_{12})[45] and cobalt protoporphyrin[46] have found occasional use in the treatment of specific tumours.

(c) Other metals

$ZnCl_2$ has found a specific application in the chemosurgical treatment of skin cancers (Moh's technique)[47,48]. Surgical removal of the tumour is followed by topical application of $ZnCl_2$ to kill remaining cancerous cells. Mercuric chloride is used as an irrigant solution for eradicating malignant cells from surgical wounds[49], and mercuric iodide has also been tried as an anti-cancer agent[50].

(ii) Chelation and cancer

No discussion of the earlier work in this field would be complete without mentioning the extensive review work of Furst[16,51]. He has postulated a relationship between chelation, carcinogens and anti-cancer agents. Later a book on the chemistry of chelation in cancer was produced, but this does not appear to have been widely read by chemists[16]. In this book Furst points out that the majority, if not all, of non-metallic carcinogens, or their proposed primary metabolic products, are potential chelating agents. This suggests the possibility that their action is due to interactions with essential metals particularly with respect to removing them from specific cellular locations; inhibition of enzymes could occur in this manner. He also remarks that some metals are known to cause cancer and that most chemotherapeutic agents used experimentally and clinically are potential metal binding agents.

Metals may be best transported into a cell in a chelated form, as most chelates have a relatively high lipid solubility and the human body is capable of concentrating not only the essential metals but also other abnormal ones. Thus a carcinogenic chelate may penetrate the cell wall and bring with it an abnormal metal (active cell transport usually involves small proteins – pinocytosis). Within the cell an equilibrium can take place between normal metal, abnormal metal, enzymes and complexing agents. Under carcinogenic conditions the abnormal metal concentration may be higher (there does not appear to be any significant evidence to support this) and the perturbation of cell metabolism could result in transformation to a neoplastic cell. Anti-cancer agents may be metal binding agents which inactivate the abnormal metal more than the normal metal in the cell. As most agents are non-selective and bind many metals, they may inactivate some of the enzymes which are necessary for rapid growth. At the same time anti-cancer metal binding agents would be likely to aid the entry of abnormal metals into normal cells and would be themselves somewhat carcinogenic. These speculations are based on general observations on carcinogens and anti-cancer agents and some seem somewhat unlikely, particularly as many anti-cancer agents have been shown to react with nucleic acids and inhibit DNA synthesis, which appears to be the general mode of action. However, Furst had the foresight to suggest using Pt^{II} and Pd^{II} complexes (1962) particularly with sulphur mustards as ligands, although he seems to have been thinking in terms of using these complexes to bring about entry of the ligands into the cells.

Kirschner et al. partially adopted Furst's suggestions and synthesised complexes with either naturally occurring biological molecules or known anti-tumour agents as the ligands[31,32,52]. They worked on the assumption that all cancers may be virally caused and that selective reaction with the virus might be possible. In fact much the same chemical arguments will apply whatever the site(s) of reaction. In this case metal ions of class "a" and "b" nature (see p. 382) were used[106], and it is suggested that reaction with a virus would release the biologically active ligand to further inhibit tumour growth. Three of the five active complexes (Table 4) consisted of Pd^{II} or Pt^{IV} bound to an S-donor ligand (6-mercaptopurine), and such strongly

TABLE 4
Screening results for some complexes with biologically active ligands (ref. 31)

Complex	Tumour[a]	Dose range[b]	Optimum T/C	Dose[c]	Toxic level[d]
$Na_2[Pt(mp)_2Cl_4].2H_2O$	S.180	44–150	7	150	> 150
	Ca.755	4–280	< 1	16	> 32
$Na_2[Pd(mp)_2Cl_2].H_2O$	S.180	100[e]	13	100	100
	Ca.755	9–36	< 1	18	9–36[g]
$Na_2[Bi(O)(mp)_2].3H_2O$	S.180	14–112	16	112	> 112
	Ca.755	0.5–46	< 1	23	45
$[Pd(butp)_3Cl]Cl$	Ca.755	3–1000	4	1000	> 1000
$[Bi(tgn)_2H_2O].3.5H_2O$	Ca.755	5–10[f]	< 1	5	5–10

[a]See Table 2. [b]Daily dose (ref. 8). [c]Dose at which optimum T/C was obtained. [d]Dose at which survivors are ≥ 83%. [e]Only one dose tested. [f]Only two doses tested. [g]Variable toxicity.

bound ligands would only be replaced with great difficulty (probably by another S-donor ligand), unless some type of enzymatic action was involved. Attachment of an active ligand to a metal may greatly aid cell transport but unless the activity is substantially better than that of the free ligand then nothing has been achieved. This appears to be the case for the complexes in Table 4. Non-transition metals (such as Bi) and some first-row transition metals will exchange their ligands fairly readily (p. 381).

The use of chelating agents to regulate the concentration of metal ions in biological systems has been practised for some three decades since the discovery of EDTA and the realisation that drugs such as 8-hydroxyquinoline act by chelation[53]. Several excellent reviews on the application of chelation to medicine are available in the proceedings of a special symposium[54]; this includes discussions on drug design of chelating agents[55–59]. The most extensive work in this area is the monograph by Albert[60] entitled *Selective Toxicity*. Many of the considerations such as metal binding ability and selectivity (thermodynamic effects and stability constants), formation constants and cell permeability are obviously relevant to the design of any metal complex drug. However, there has been much less discussion concerning kinetic factors.

C. PLATINUM COMPOUNDS

As mentioned in the Introduction, the discovery of anti-tumor activity among platinum complexes has been the most important factor in stimulating a new interest in metal complexes as a potential class of anti-tumour drugs. Most of the systematic work carried out so far has been in this area and for this reason the remainder of the review will be dominated by studies on platinum compounds.

(i) Discovery of biological activity

Whilst investigating the effects of an electric field on the growth processes in bacteria, Rosenberg and his co-workers found that cell division was inhibited but cell growth was not affected[6]. The passage of current via platinum electrodes immersed in a nutrient medium (known as C medium[61] and composed of NH_4Cl 2 g/l; Na_2HPO_4 6 g/l; KH_2PO_4 3 g/l; NaCl 3 g/l; $MgCl_2$ 0.01 g/l; Na_2SO_4 0.026 g/l) containing *Escherichia coli* bacteria, resulted in the formation of long filamentous bacterial rods (Fig. 3). Further tests showed that this response was limited to gram-negative bacteria. These effects had only been noted previously for physical agents such as UV light[62], osmotic pressure or temperature changes[63], transfer to an unaccustomed medium, and a few organic compounds including methylene blue[64] and penicillin[65]. A long series of control experiments showed that the current itself was not causing the filamentous growth, but it was causing some 10 p.p.m. of platinum to dissolve electrolytically into the C medium from the platinum electrodes. The species formed during the electrolysis was identified as $[PtCl_6]^{2-}$, which is present in part as the

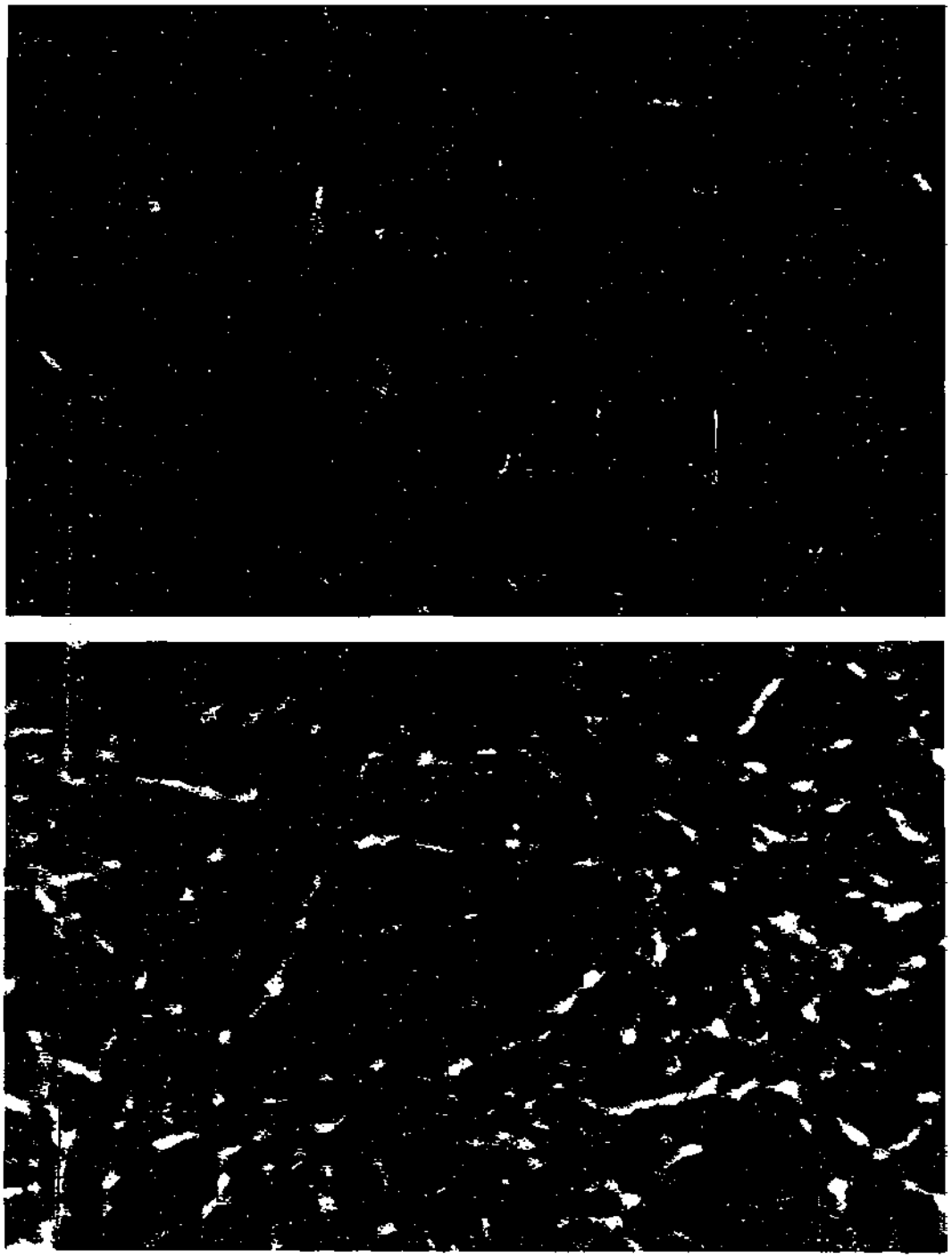


Fig. 3. Phase contrast photomicrographs of *E. coli B* (× 600). Above are normal bacteria grown in chemically defined medium and below are filamentous bacteria grown in the same medium but incorporating 10 p.p.m. of *cis*-$[Pt(NH_3)_2Cl_2]$ (ref. 72).

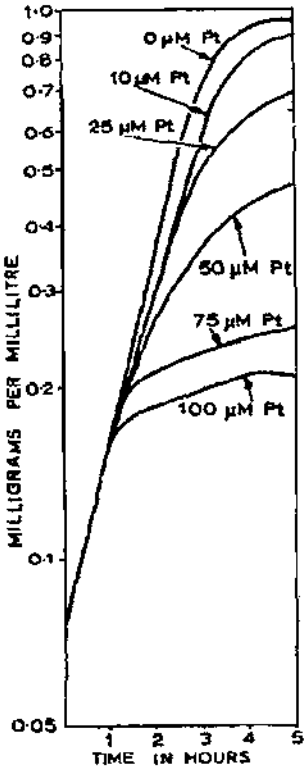


Fig. 4. Filamentous growth curve as measured by turbidity of *E. coli* bacteria in test tubes in a chemically defined medium. Concentrations of *cis*-$[Pt(NH_3)_2Cl_2]$ vary from 10–100 μM (2–20 p.p.m.) and after 1 h all the bacteria are in the form of filaments (ref. 72).

ammonium salt in C medium. Fresh solutions of $(NH_4)_2[PtCl_6]$ are bacteriostatic and inhibit cell growth at these concentrations (~ 10 p.p.m.). Van Camp and co-workers noticed that aged solutions (2–3 days) were very effective in producing filaments at low platinum concentrations[7]. Spectroscopic and ionophoretic studies confirmed the photochemical reaction[7]

$$[PtCl_6]^{2-} + n\ NH_4^+ \rightarrow cis\text{-}[PtCl_{6-n}(NH_3)_n]^{(2-n)-} + n\ H^+ + n\ Cl^-$$

which in the time period studied did not proceed much beyond the $n = 2$ stage. The pentachloro species, $[PtCl_5(NH_3)]^-$, is neither an effective growth inhibitor nor cell division inhibitor, but as it readily converts to the neutral species in the precence of C medium and light it does appear to force filamentous growth. The neutral species *cis*-$[Pt(NH_3)_2Cl_4]$ is a potent inhibitor of cell division while having only a small inhibitory effect on the growth rate (Fig. 4). Testing of synthesised *cis* and *trans* isomers confirmed that the *cis* species is biologically active while the *trans* isomer has relatively little effect on the cell growth processes. At the same time the corresponding *cis* and *trans* Pt^{II} species, $[Pt(NH_3)_2Cl_2]$, were tested and again only the *cis* compound caused filamentation (Fig. 4). This is also likely to be formed in small quantities during the electrolytic and photochemical reactions due to reduction of *cis*-$[Pt(NH_3)_2Cl_4]$.

TABLE 5
Distribution of ^{191}Pt among the various classes of cellular compounds extracted from bacteria (expressed as a percentage of radioactivity) (ref. 66)

Fraction	Aged $(NH_4)_2[PtCl_6]$ [a]			Fresh $(NH_4)_2[PtCl_6]$
	E. coli	*B. cereus*	*S. aureus*	*E. coli*
Metabolic intermediates	19	74	60	1
Lipids	6	2	1	3
Nucleic acids	30	19	20	1
Cytoplasmic proteins	45	5	19	96

[a] Contains mainly *cis*-$[Pt(NH_3)_2Cl_4]$.

(ii) Bacterial studies

Renshaw and Thomson[66] studied the distribution of platinum (from irradiated $(NH_4)_2[PtCl_6]$) in *E. coli* and two gram-positive bacteria *B. cereus* and *S. aureus* (Table 5). In the latter case most of the platinum was bound to metabolic intermediates, whereas in *E. coli* it was distributed amongst the cytoplasmic proteins and nucleic acids. A clue to the mode of action was given by a comparison with the distribution of platinum from a fresh $(NH_4)_2[PtCl_6]$ solution, where nearly all the platinum was in the cytoplasmic protein and very little was associated with the nucleic acid (Table 5).

Other Group VIII metal compounds were tested against bacteria and some ruthenium and rhodium compounds were found to cause filamentous growth in *E. coli*[6,67] (Table 6) but not to the same extent as for the platinum compounds. Other types of filamentous growth can be reversed and break up of the filaments (cytokinesis) achieved by the addition of various agents such as pantoyl lactone and some divalent cations[68,69]. In this case none of these agents had any effect and cytokinesis could only be initiated by removal or decrease of the platinum in the culture. This suggested that the cause of Pt-induced inhibition of cell division might differ from those previously reported.

Another aspect of the bacterial activity was reported by Reslova et al.[70,71], who found that lysogenic strains of *E. coli* (i.e. *E. coli* which have previously been infected by bacteriophage, from which genetic material has been incorporated into the bacterial cell and become repressed and not normally detectable) can be induced by the platinum compounds to develop partial or complete viruses leading to lysis (destruction) of the cell (Fig. 5). Other agents such as UV and X-rays and chemicals, including nitrogen mustard and other known anti-tumour agents, are also known to have this effect[72].

(iii) Anti-tumour studies

The property of inhibiting cell division but not cell growth suggested that these compounds might have anti-tumour properties and this was emphasised by the fact

TABLE 6
The effects of some Group VIII complexes on bacterial growth (*E. coli* B – refs. 6,7,109)

Complex	Bacteriostatic concentration (μg/ml)	Filament-inducing concentration (μg/ml)	Filaments (%)	Elongation
Rhodium				
$(NH_4)_3[RhCl_6]$		20–30	75	5–25×
$K_3[Rh(NO_2)_6]$		20–60	10	3–5×
$RhCl_3$ (aq)*		30–100	75	5–25×
trans-$[Rh(NH_3)_4Cl_2]NO_3$		25	10	0–5×
mer-$[Rh(NH_3)_3Cl_3]$		25	[a]	5–10×
trans-$[Rh(py)_4Cl_2]Cl.5H_2O$ [b]	20	2.5	[a]	5–10×
cis- and *trans*-$[Rh(en)_2Cl_2]NO_3$		Inactive		
Ruthenium				
$(NH_4)_3[RuCl_6]$		15–30	10	5–20×
$K_2[Ru(NO)Cl_5]$		40–100	50	5–10×
$[Ru(NH_3)_3Cl_3]$	> 25	5	[a]	> 25×
$RuCl_3$ (aq)		Inactive		
Iridium				
mer-$[Ir(NH_3)_3Cl_3]$		Inactive		
$K_3[Ir(NO_2)_6]$		Inactive		
$(NH_4)_2[IrCl_6]$		Bacteriocidal		
Palladium				
$[Pd(NH_3)_2ox]$	> 0.25			
[Pd(en)ox]	> 0.25			
$(NH_4)_2[PdCl_4]$		Bacteriocidal		
Others				
$[UO_2(CH_3CO_2)_2]$		25–75	5	3–5×
$(NH_4)_2[OsCl_6]$		Bacteriocidal		
$NiCl_2$		Bacteriocidal		
$CoCl_2$		Bacteriocidal		
$[Co(NH_3)_6]Cl_3$		Inactive		
$[Ni(NH_3)_6]Cl_2$		Inactive		

[a]Not estimated. [b]For more data see ref. 111. *Widely differing results have been obtained for various samples of hydrated rhodium trichloride.

that other anti-tumour agents (e.g. alkylating agents, actinomycin D) also caused elongation and lysis in lysogenic bacteria. Initially four compounds (*cis*-$Pt(NH_3)_2Cl_4$, *cis*-$[Pt(NH_3)_4Cl_2]$, $[Pt(en)Cl_4]$, $[Pt(en)Cl_2]$) were tested against Sarcoma 180 in the ICR strain of mice and were found to be effective in inhibiting the tumour growth[1], leaving some animals tumour-free (Table 7). As had been predicted by the bacteriological results, neither of the *trans* isomers (Pt^{II} or Pt^{IV}) showed any appreciable activity. The two *cis* compounds were submitted to the U.S. National Cancer Institute and were screened against L.1210 leukaemia in mice[1]. They showed potent

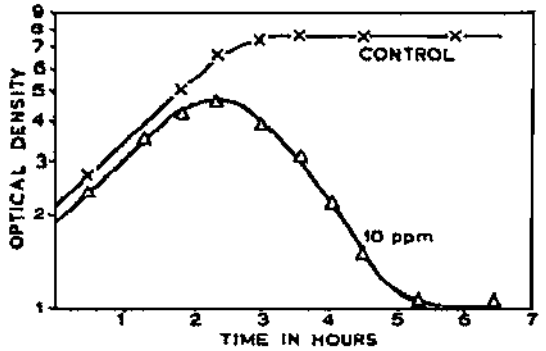


Fig. 5. Growth curve for a lysogenic strain of *E. coli* grown in the presence of a 10 p.p.m. solution of *cis*-$[Pt(NH_3)_2Cl_2]$. After 3 h the induced viruses cause complete lysis of the bacteria. Most anti-tumour complexes show this property, whereas many compounds which are not appreciably active cause simple filamentation (ref. 72).

anti-tumour activity and effected several cures with single injections at the therapeutic dose of 8 mg per kg of body weight. Rosenberg and Van Camp went on to show that *cis*-$[Pt(NH_3)_2Cl_2]$ was capable of regressing large solid Sarcoma 180 tumours (8 days old) in Swiss white mice[2]. A single intraperitoneal dose of 8 mg per kg caused the complete regression of at least 60% of the tumours. As with most cures of transplanted tumour systems, this produced long-term immunity to this particular tumour. As *cis*-$[Pt(NH_3)_2Cl_2]$ appeared to be the most potent of the original compounds it has now been extensively tested against many other transplantable tumours, and has been shown to have a wide spectrum of activity (Table 8). It is now undergoing extensive human clinical trials in the U.S.A. under the auspices of the National Cancer Institute. Although these trials are far from completion, the preliminary clinical results are promising and show definite tumour growth inhibition[73,74]. These results coupled with some of the earlier reports, suggest that inor-

TABLE 7
The original anti-tumour results for platinum complexes (ref. 1)

Complex	Tumour[a]	Dose range[g]	Optimum *T/C* (%I.L.S.)	Dose[b]
cis-$[Pt(NH_3)_2Cl_2]$	S.180	0.5–2[c]	1.8	2.0
	L.1210	1.25[d]	87	1.25
		5–10[e]	> 83[f]	10
cis-$[Pt(NH_3)_2Cl_4$	S.180	2.5–10[c]	29	10
	L.1210	2.5[d]	49	2.5
$[Pt(en)Cl_2]$	S.180	1.25–5[c]	3.6	5.0
$[Pt(en)Cl_4]$	S.180	0.6–5.0[c]	20	5.0

[a]See Table 2. [b]Dose at which optimum *T/C* (I.L.S.) was registered. [c]Daily for ten days. [d]Daily for 9 days. [e]Single dose only. [f]3 of 10 mice were alive and tumour-free at termination of test. [g]All doses in mg/kg.

TABLE 8
Some tumour types tested in animals with *cis*-$[Pt(NH_3)_2Cl_2]$[a]

Tumour	Host	Best results
Sarcoma 180 (solid)	Mice (Swiss white)	*T/C*, 2–10
Advanced S.180	Mice (Swiss white)	100% cures
Dunning leukaemia (advanced)	Rats (Fisher 344)	100% cures
Walker 256 carcinosarcoma	Rats (Fisher 344)	100% inhibition (T.I. = ~ 3)
Rous sarcoma (advanced)	Chicks	95% total regression
ADJ/PC6 (plasma cell)	Mice (BALB/c)	100% inhibition (T.I. = 8.1)
Mammary carcinoma (DMBA-induced)	Rats (Sprague–Dawley)	60% total regression; 3/11 cures
Reticulum cell sarcoma	Mice	I.L.S., 180%
Ehrlich ascites tumour	Mice (BALB/c)	I.L.S., ~ 300%
Leukaemia L.1210	Mice (BDF_1)	I.L.S., 380%
Lewis lung carcinoma	Mice (BDF_1)	100% cures
B-16 Melanocarcinoma	Mice (BDF_1)	I.L.S., 222%

[a]This table was compiled by Dr. B. Rosenberg, Michigan State University.

ganic coordination compounds form a new class of anti-tumour agents which is still largely unexplored. This should allow the considerable knowledge of coordination chemistry which has been built up over the last twenty years to be applied to this somewhat unfamiliar field. Two major areas will be (*i*) synthetic studies with a view to determining what relationships exist between chemical structure and anti-tumour activity and with a view to finding better anti-tumour agents, and (*ii*) physico-chemical studies on the interaction of coordination complexes with metabolites and macromolecules with a view to determining the cell binding sites. As Thomson et al. have recently pointed out, transition metals, in particular the heavier ones like platinum, can make excellent probes in a biological system [71]. Research in both these areas is in progress.

D. ANALOGUES OF *cis*-$[Pt(NH_3)_2Cl_2]$; FACTORS INFLUENCING ANTI-TUMOUR ACTIVITY

A reasonable starting supposition is to assume that if a complex is to show anti-tumour activity then its reactivity should fall between certain undefined limits. Thus it should be sufficiently inert so as not to react completely with the many other available metabolic sites, yet it must be sufficiently reactive to bind to some extent at the site(s) of anti-tumour action (which is likely to be nuclear DNA). The extent of the binding will obviously depend on the nature of this final product. Many metabolic intermediates and macromolecules are capable of reacting with a coordination complex – for example almost every compound in the Krebs cycle (tricarboxylic acids) is a potential ligand. Indeed, one of the keys to the biological chemistry of all the essential transition metals is likely to be the determination of formation constants of such complexes.

It is important to emphasise that the biological results obtained for a compound apply only to the particular tumour system used (including the breed of animals) and do not necessarily indicate activity against other tumours. It is good practice for institutes to use different tumours as discussed earlier, and in the early stages of screening we can consider that activity against any known tumour system indicates that the compound shows potential even though it may be shown subsequently that the activity is limited to one or two systems only.

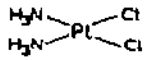
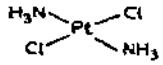
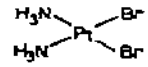
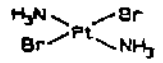
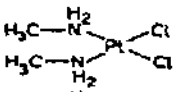
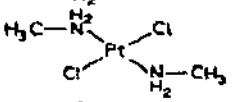
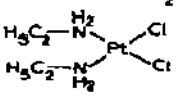
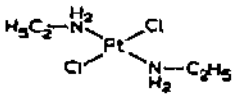
(i) A comparison of cis- *and* trans- *complexes*

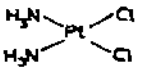
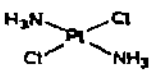
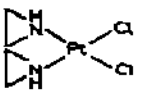
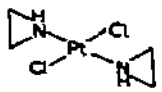
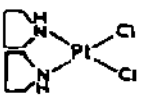
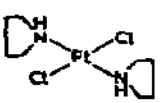
An obvious similarity between Rosenberg's original active complexes is the *cis* arrangement of the chloride ligands. This feature has remained constant in the two major studies reported to date[75-77] and where a *trans* isomer exists and has been tested, it has been found to be inactive (and usually non-toxic) in comparison to an active *cis* isomer (Table 9). Thus for platinum complexes this had led to a concentration of effort on *cis* complexes of the general type *cis*-$[PtA_2X_2]$, where A_2 is two monodentate or one bidentate amine ligand and X_2 is two monodentate or one bidentate anionic ligand. A notable feature of the chemistry of these complexes is that the *trans* isomers are consistently more reactive than their *cis* analogues. Thus *trans*-$[Pt(NH_3)_2Cl_2]$ aquates approximately four times faster[78] than *cis*-$[Pt(NH_3)_2Cl_2]$, and undergoes ammonation some thirty times faster (although the corresponding *cis* equilibrium constants for these reactions are larger)[79]. Thus *trans* compounds are likely to react more quickly and with a wider variety of body constituents and should be rather less specific in their action than *cis* compounds. Indeed distribution and excretion studies using ^{195m}Pt-enriched $[Pt(NH_3)_2Cl_2]$ isomers indicate that the *cis* is initially excreted faster (although the whole body retention after 5 days is comparable), and the relative levels of the isomers in the blood indicates a higher initial concentration for *trans* (~ 3×) coupled with a higher retention after 5 days[80,81]. Since only *cis* compounds have the potential to form chelates, this might imply that the anti-tumour activity is largely associated with a chelating interaction (p. 393). Chemical studies on the $[Pt(NH_3)_2Cl_2]$ isomers have clearly established that the chlorides are the reactive ligands[82,83]. Although NH_3 is low in the *trans*-effect series, the Pt—NH_3 bond is very stable[84] and ranks close to CN^- in affinity for Pt^{II}. This stability, coupled with the fact that NH_3 is a poor leaving group, dominates the chemistry of these complexes, overriding the slightly greater *trans*-effect of the chlorides (which are good leaving groups).

(ii) Variation of X in cis-$[PtA_2X_2]$

Compounds of the type *cis*-$[Pt(NH_3)_2X_2]$ have been prepared via the aquo species, $[Pt(NH_3)_2(H_2O)_2]^{2+}$, which is formed when *cis*-$[Pt(NH_3)_2Cl_2]$ is reacted with silver nitrate[85]. The new complex is produced by addition of the appropriate anion (X =

TABLE 9
Comparison of activities for *cis* and *trans* isomers

Complex		Solvent	Dose range (mg/kg)	Dose response	Toxic level (mg/kg)	T/C	Dose (mg/kg)
Sarcoma 180 (ref. 75)							
	*cis*	S	0.5–20	+	9	1	8
	*trans*	S	2.5–40[b]	–	> 40	85	2.5–40
	*cis*	B	5–20	+	15	30	14
	*trans*	B	10–40	–	> 40	110	10–40
	*cis*	S	10–30	+	12–20	25	15[a]
	*trans*	S	5–100	–	25	100	5–20
	*cis*	S	5–50[b]	+	45	14	40
	*trans*	SS	5–20	–	> 20	105	5–20

Complex		Solvent	Dose range (mg/kg)	Dose response	L.D$_{50}$ (mg/kg)	L.D$_{90}$ (mg/kg)	T.I.
ADJ PC6 plasma cell tumour (ref. 76)							
	*cis*	A	0.1–40	+	13.0	1.6	8.1
	*trans*	A	6–800	–	27.0	> 27.0	< 1.0
	*cis*	A		+	56.5	2.6	21.7
	*trans*	A	2.5–160	–	18.0	> 18.0	< 1.0
	*cis*	A		+	240	17.5	13.7
	*trans*	A		–	72	> 72	< 1.0

[a]Only 66 per cent survivors. [b]Slurry at higher concentrations. [c]Sporadic toxicity over this range.

TABLE 10
Variation of X in *cis*-$[Pt(NH_3)_2X_2]$ with Sarcoma 180 tumour (ref. 75)

X	Solvent	Dose range (mg/kg)	Dose response	Toxic level (mg/kg)	*T/C*	Dose (mg/kg)
NO_3^-	W	6–12	–	7[c]	54	6
NO_3^-	S	2.5–12	+	11	8	10
H_2O[d]	W	2–20	–	5[c]		
Cl^-	S	0.5–20	+	9	1	8
Br^-	B	5–20	+	15	30	14
Br^-	S	2–6[b]	+	5–6[b]	13	5
I^-	W.S.	10–25	–	> 25	110	10–25
SCN^-	S	5–100[a]	–	~ 50	70	20–35
NO_2^-	S.S.	5–100	–	> 100	99	5–100

[a]Slurry at higher concentrations. [b]Daily injections for 9 days. [c]Highly toxic – convulsions. [d]Cationic complex ion (2+).

Br, I, SCN, NO_2, NCO; X_2 = ox, mal (substituted malonates)[75].

Table 10 shows the effect of varying X on the activity against Sarcoma 180 in Swiss white mice[75]. From the general chemistry of platinum ammine complexes, it is likely that biological reactions will occur with X as the leaving group. The order of leaving ability has been established for the reaction[83]

$$[Pt(dien)X]^+ + py \rightarrow [Pt(dien)py]^{2+} + X^-$$

where the order of decreasing rate constants is $X = NO_3^- > H_2O > Cl^- > Br^- > I^- > SCN^- > NO_2^-$. The spread in rates for this series of reactions is about 10^6, showing that the leaving group, and consequently bond breaking, has a substantial effect on the reaction rate. Even though the nature of the incoming moieties in the biological reactions cannot be defined (even if one specific DNA reaction is essential for anti-tumour activity many other reactions will obviously occur to some extent), the

NOTE:

For Tables 9–21.

(i) The solvent is indicated by: S = saline (0.15 *M* NaCl), S.S. = saline slurry, W = water, W.S. = water slurry, B = sodium bromide (0.04 *M*), B.S. = sodium bromide slurry.

(ii) Dose response is termed positive (+) for cases where a consistent decrease in *T/C* value occurred with increasing doses up to the toxic level. Where the decrease of *T/C* with dose was inconsistent (but values ≤ 50 were obtained) the response is termed ±.

(iii) Toxic level is the highest dose at which survivors are ≥ 83%.

(iv) The *T/C* value is the lowest obtained for compounds with a positive dose response. In cases of marginal or negative response the *T/C* values are averages over the dose range given in the final column.

(v) The final column shows the dose at which the *T/C* was obtained.

screening results reflect the order of leaving ability. Where the ligands X are readily replaced (H_2O, NO_3^-) the complexes only show activity when administered in saline, when the chloro species has been partially reformed prior to and during innoculation. When injected in aqueous solution they show a high and immediate toxicity which appears to be due to action at the neuromuscular junction (p. 379). The nitrato species is rapidly hydrolysed[85] in water to give $[Pt(NH_3)_2(H_2O)_2]^{2+}$ in equilibrium with $[Pt(NH_3)_2(H_2O)(OH)]^+$ (pK_a 5.63) but very little[86] $[Pt(NH_3)_2(OH)_2]$ (pK_a 9.25) at neutral pH. Recent studies on Fowl Pox virus in embryonating eggs indicate that this solution has potential as an anti-viral agent[87]. Compounds with ligands of intermediate leaving ability (Cl^-, Br^-) show considerable anti-tumour activity. This activity parallels the leaving ability, with chloride more effective than bromide, while *cis*-$[Pt(NH_3)_2Br_2]$ requires a higher dose (in terms of Pt) to be effective and has a higher toxic level. The iodide follows the trend and is inactive up to high doses; it is insoluble and requires a slurry injection. The same order of halogen complex activity has been observed for other *cis* amine compounds[75]. The strongly bonded $-SCN$ and $-NO_2$ ligands give compounds with no anti-tumour effect while the toxic doses are relatively high. It seems likely that these ligands are so strongly attached that little or no reaction takes place within the animal's body.

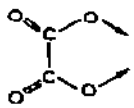
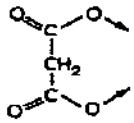
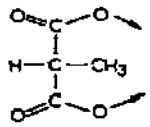
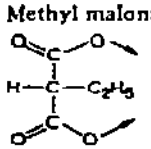
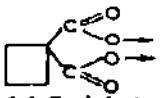
Thus the active compounds lie within a "window of reactivity" which is at least partially defined by the nature of the Pt–X bond. Complexes with mixed monodentate anionic ligands, i.e. *cis*-$[Pt(NH_3)_2XY]$ (X, Y = Cl, Br, I, NO_2, SCN) have been tested but the activity was not enhanced and the toxicity was high and rather variable[75].

Complexes with chelated dicarboxylate ligands (oxalate and malonates) show considerable activity[75] against Sarcoma 180 (Table 11). The malonate, $[Pt(NH_3)_2$-mal], has also been successfully tested against the ADJ/PC6A tumour[76]. This compound is active against L.1210[88] and against advanced S.180 tumours[89]. Except for the oxalate these are new complexes and consequently no kinetic data have been reported. However, they are expected to be relatively inert to substitution[90,91] and this has been confirmed for aqueous solutions by UV spectral and conductivity studies[75]. Thus it is difficult to explain the anti-tumour effect in terms of leaving ability. Studies on their reactions with chelating nitrogen ligands, especially those found in DNA, are highly desirable. If the rates of replacement are as slow as might be expected ($K_2[Pt(ox)_2]$ shows a rate constant[90,91] for oxalate exchange of the order of 10^{-7}) then enzymatic removal may be operating. Free malonate and oxalate ions can be bound by several cellular enzymes (e.g. succinate dehydrogenase) and a study of in vitro reactions with a selection of pure enzymes is desirable. Malonic acid and several of its derivatives have been shown to have some tumour-inhibiting properties[91–95] against several different mouse systems although they were inactive against S.180 and some human neoplasms[90,97]. Complexation of malonate is known to activate the methylene group protons[98]. Enzyme-controlled in situ release of the reactive platinum moiety may well be advantageous regardless of the carcinostatic

effect of the free ligand. It may be significant that carboxylate or closely related ligands have often been involved in older reports of metal complex activity (Section B, e.g. butylphthalates, ascorbates, dithioxamides, malates). A systematic study of carboxylates in relation to anti-tumour (or general biological) properties would be extremely interesting.

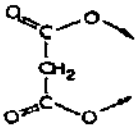
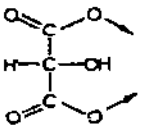
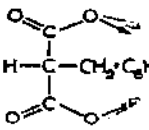
The $[Pt(en)X_2]$ series has also been investigated using the Sarcoma 180 system (Table 12)[75]. Again the diaquo species is toxic and gives rise to neuromuscular disturbances. The effectiveness of the halogeno complexes falls in the order Cl > Br > I. The malonate and substituted malonate complexes show good activity (comparable to the chloride), but the oxalato complex is anomalous and shows extremely high toxicity, with neuromuscular effects similar to those found with the diaquo species but somewhat worse. Conductivity and UV/vis spectral studies indicate that

TABLE 11
Activity of ammine complexes with chelated dicarboxylate ligands X in $[Pt(NH_3)_2X]$

X	Solvent	Dose range (mg/kg)	Dose response	Toxic level (mg/kg)	T/C	Dose (mg/kg)
Sarcoma 180 (ref. 75)						
	DMSO (slurry)	5–20	+	16–20	9	15
	W.S.	12–18	+	17	24	14–16
	W	10–20[c]	+	10	0	10[a]
Oxalate	W	0.5–6[b]	+	3–4	25	2.5
	W.S.	10–60	+	35	7	30
	W	5–24[c]	+	24	28	20–24
	W	1–7[b]	+	8	12	7
Malonate						
	W	10–80	+	65	7	60
Methyl malonate						
	W	30–80	+	> 80	17	70–80
Ethyl malonate						
	W	20–160	+	150	18	120
1,1-Cyclobutane dicarboxylate						

(continued)

TABLE 11 (continued)

X	Solvent	Dose range (mg/kg)	Dose response	$L.D_{50}$ (mg/kg)	$I.D_{90}$ (mg/kg).	T.I.
ADJ/PC6 plasma cell tumour (refs. 76, 100)						
 Malonate	A	1–320	+	225	18.5	12.2
 Hydroxymalonate (tartronate)	A	2–1500	+	150	4.9	30.6
 Benzyl malonate	A	1–1500	+	150	1.85	81.1
1,1-Cyclobutane dicarboxylate	A	4–256	+	180	14.5	12.4

[a] 66% survivors only. [b] Daily injections for 9 days. [c] Multiple injections due to low solubility.

TABLE 12

Changes in activity on varying X in $[Pt(en)X_2]$ and $[Pt(en)X]$ for Sarcoma 180 in Swiss white mice (ref. 75)

X	Solvent	Dose range (mg/kg)	Dose response	Toxic level (mg/kg)	T/C	Dose (mg/kg)
H_2O[c]	W	2–16	±	5[b]	25	4
Cl^-	S	2.5–32	+	14	27	12
Cl^-	S	1–10[a]	+	~6	4	5
Br^-	S	8–16	+	> 16	31	16
I^-	S.S.	5–40	–	> 40	82	5–40
SCN^-	W	10–40	–	> 40	83	10–40
NO_2^-	S.S.	25–100	–	> 100	71	75–100
ox^{2-}	W	0.25–16	–	35	73	0.25–2
mal^{2-}	W	5–80	+	45–60	18	40
mal^{2-}	S	45–60	±	> 60	41	50–60
mal^{2-}	S	5–20[a]	+	6–10	24	5
$Memal^{2-}$	W	30–90	+	> 90	4	90
$Etmal^{2-}$	W.S.	40–120	±	> 120	51	90–120

[a] Daily injections for 9 days. [b] Highly toxic – convulsions. [c] Cationic complex ion (2+).

aged solutions of the oxalato complex have not hydrolysed to any appreciable extent, and the equivalent dose of sodium oxalate does not harm the animals. $[Pt(NH_3)_2ox]$ did not give these symptoms when injected as a slurry although multiple injections of an aqueous solution (solubility is 10 mg/100 ml at 37°C) did produce some minor signs of neuromuscular toxicity, but these were mild compared with those elicited by the corresponding ethylenediamine complex. This again indicates some sort of in vivo biological activation of oxalate ligands. In other amine systems, the halogeno complex activity falls in the same order as described above. Generally if a chloro complex exhibits activity the corresponding malonate (and usually oxalate) also shows a good response (against S.180). Some apparently dimeric pyrophosphate complexes $[(PtA_2)_2P_2O_7]$ have been prepared but show little sign of activity[75] (see page 398).

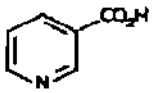
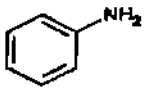
(iii) Variation of A in cis-$[PtA_2X_2]$

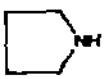
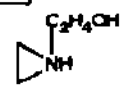
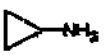
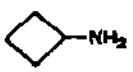
Whereas the ligands X will largely determine the overall reactivity of these complexes, the nature of the ligand A should modify this in a secondary manner due to different steric, electronic and basic properties. In cases of extreme steric hindrance the rate of substitution can be greatly lowered. However, the A groups have a primary effect on the anti-tumour property (Tables 13 and 14) and this is difficult to correlate with any chemical reactivity effects. This is particularly apparent in the ADJ/PC6A test results[76].

In the S.180 system, complexes with primary amines ($R.NH_2$; R = alkyl) retain activity although at an apparently reduced level compared with the parent ammine. With secondary amines (R_2NH; R = Me, Et) the response was only marginally apparent[75]. On the plasma cell tumour, methylamine gave rise to greatly reduced activity but with no change in toxicity. Effects like this indicate that toxicity may not be so closely related to anti-tumour activity as to prevent the existence of other compounds with a low toxicity and a high activity, at least against this tumour[76]. In fact complexes with the heterocyclic amines, ethyleneimine and pyrrolidine, are two to three times more selective than the parent compound. However, relatively small changes to morpholine and *N*-2-hydroxyethyl-ethyleneimine lead to complete loss of activity[76]. The ethyleneimine complex has shown no activity[99] towards S.180. Although ethyleneimines are anti-tumour agents in their own right[4], the ligand is stabilised on coordination and it is unlikely that the ethyleneimine itself plays a role in the response.

The most interesting results have come from complexes with alicyclic amines. The sequence from cyclopropylamine through cyclooctylamine gives excellent results against the ADJ/PC6 system, with those for the cyclopentylamine and cyclohexylamine particularly outstanding[76]. These have the best therapeutic indices ever reported for this tumour system. The indices appear to rise to a maximum at the C_5 and C_6 compounds.

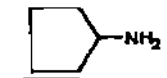
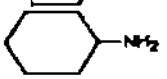
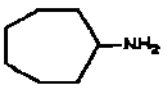
TABLE 13
Changes in activity on varying A in *cis*-$[PtA_2Cl_2]$

A	Solvent	Dose range (mg/kg)	Dose response	Toxic level (mg/kg)	T/C	Dose (mg/kg)
Sarcoma 180 (ref. 75)						
H_3N	S	4–15	+	9	3	8
CH_3NH_2	S	10–30	+	12–20[b]	14	14[a]
$(CH_3)_2NH$	S	30–150	+	~ 100	25	80
$C_2H_5NH_2$	S	5–50	+	~ 40	14	40
$(C_2H_5)_2NH$	S.S.	15–60	–	> 60	75	60
$HOC_2H_4NH_2$	S	20–225	+	~ 125	22	125
$i\text{-}C_3H_7NH_2$	S.S.	20–50	±	> 50	33	30
	W.S.	5–40	–	> 40	94	5
	W[c]	4–80	±	> 80	51	40–60
	S.S.	10–50	+	> 50	~ 33	10–20

A	Solvent	Dose range (mg/kg)	Dose response	$L.D_{50}$ (mg/kg)	$I.D_{50}$ (mg/kg)	T.I.
ADJ/PC6 plasma cell tumour (ref. 76)						
NH_3	A	0.1–40	+	13.0	1.6	8.1
CH_3NH_2	A		–	18.5	18.5	1.0
$ClC_2H_4NH_2$	A		+	45.0	17.5	2.6
	A	2.5–160	+	56.5	2.6	21.7
	A	3–200	+	141	10.8	13.1
	A		–	90	> 90	< 1.0
	A		–	18	> 18	< 1.0
	A	1–80	+	56.5	2.3	24.6
	A	6–750	+	90	2.9	31.0

(continued)

TABLE 13 (continued)

A	Solvent	Dose range (mg/kg)	Dose response	L.D$_{50}$ (mg/kg)	I.D$_{90}$ (mg/kg)	T.I.
	A	1–3200	+	565.6	2.4	235.7
	A	1–3200	+	> 3200	12	> 267
	A	5–625	+	> 625	18	> 35

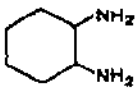
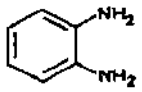
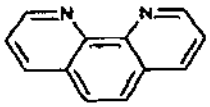
[a]66% survivors. [b]Variable toxicity. [c]Injected as sodium salt.

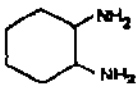
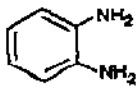
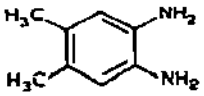
TABLE 14
Changes in activity on varying A in [PtACl$_2$]

A	Solvent	Dose range (mg/kg)	Dose response	Toxic level (mg/kg)	*T/C*	Dose (mg/kg)
Sarcoma 180 (ref. 75)						
$H_2N.CH_2.CH_2.NH_2$ Ethylenediamine	S	2–32	+	16	27	12
$HN(CH_3).CH_2.CH_2.NH_2$ *N*-Methylethylene-diamine	S	7.5–20	±	10–15	51	15[a]
$HN(CH_3).CH_2.CH_2.NH(CH_3)$ *N,N'*-Dimethylethylene-diamine	S	20–80	±	25–35[b]	26	30
$H_3C.N(CH_3).CH_2.CH_2.NH_2$ *N,N*-Dimethylethylene-diamine	S.S.	25–100	–	75–100	60	25–75
$H_3C.N(CH_3).CH_2.CH_2.NH(C_2H_5)$ *N,N*-Dimethyl-*N'*-ethylethylenediamine	S.S.	50–125	±	~ 120	62	100
$HN(C_2H_5).CH_2.CH_2.NH(C_2H_5)$ *N,N'*-Diethylethylene-diamine	S.S.	75–225	–	> 225	96	75–225

(continued)

TABLE 14 (continued)

A	Solvent	Dose range (mg/kg)	Dose response	L.D$_{50}$ (mg/kg)	I.D$_{90}$ (mg/kg)	T.I.
$H_5C_2.N(C_2H_5).CH_2.CH_2.NH_2$ *N,N*-Diethylethylenediamine	S	10–100	±	> 100	54	75–100
$H_2N.CH_2.CH(NH_2).CH_3$ 1,2-Propylenediamine	S.S.	5–20	±	8–12[b]	62	12[a]
$H_2N.CH_2.CH_2.CH_2.NH_2$ 1,3-Propylenediamine	S.S.	8–30	±	10–15[b]	58	15–30
 1,2-Diaminocyclohexane	S.S.	10–30	±	20–35[b]	62	15–30
 *o*-Phenylenediamine	S.S.	20–80	–	> 80	120	20–80
 *o*-Phenanthroline	S.S.	10–30	–	15	69	10

A	Solvent	Dose range (mg/kg)	Dose response	L.D$_{50}$ (mg/kg)	I.D$_{90}$ (mg/kg)	T.I.
ADJ/PC6 plasma cell tumour (ref. 76)						
 1,2-Diaminocyclohexane	A	0.3–40	+	141	2.1	6.9
 *o*-Phenylenediamine	A	0.6–80	+	48	2.4	20.4
 4,5-Dimethyl-*o*-phenylenediamine	A	12–1500	+	680	< 12	> 56.7

[a]66% survivors. [b]Variable toxicity.

TABLE 15
Animal testing results for complexes of type *cis*-$[PtL_2Cl_2]$
L_2 = two non-N-donor ligands or a mixed N- and O-donor chelate (ref. 75)[c]

L	Solvent	Dose range (mg/kg)	Dose response	Toxic level (mg/kg)	*T/C*	Dose (mg/kg)
PPh_3	S.S.	25–100	±	> 100	~53	25–100
	S.S.–DMSO[b]	40–160	±	> 160	~78	40–160
DMSO	S	6–12	–	> 12	~86	6–12
Et_2S	DMSO	5–40	–	> 40	~67	5–20
gly[a]	S	10–200	–	125–150	79	40–100

[a]Anionic complex (–). [b]Slurry in 10% DMSO–saline mixture. [c]Sarcoma 180 in Swiss white mice.

However, when the cyclohexylamine derivative was tested against other tumour systems (S.180 (ref. 99), L.1210 and Walker 256 carcinosarcoma [100]) the activity was low, suggesting that its action was very tumour-specific. Results for the cyclopentylamine further complicate the situation as they indicate that it is active against a wider number of systems [100]. When the alicyclic ring is further removed from the nitrogen donor, as with cyclohexylmethylamine, the activity is completely lost [101]. Those aromatic amines which have been tested generally show no promise. An exception is *o*-phenylenediamine (and 4,5-dimethyl-*o*-phenylenediamine) which shows relatively good selectivity [76] against the ADJ/PC6 (Table 15). The 1,10-phenanthroline complex is inactive against S.180 and ADJ/PC6. The aniline derivative is marginally active against S.180 but the pyridine one was inactive at the levels tested [75]. However, the latter compound was studied by Gale et al. and tested against the Ehrlich ascites tumour in Balb/$\bar{c}$ mice. A significant increase in lifespan was noted (130–170% at 50–100 mg/kg in a single dose) but the compound was considerably less potent against this tumour than the corresponding ammine [102]. Filamentous growth of *E. coli* was also reported. The binding characteristics with living and non-living systems were studied using the compound prepared with tritiated pyridine [103] (p. 389).

In the case of bidentate aliphatic amines, the ethylenediamine complex shows a good response against S.180 (as originally reported by Rosenberg et al. [1]), ADJ/PC6 (ref. 76) and Walker 256 carcinosarcoma tumours [100]. Alkyl-substituted species have been tested against S.180 (Table 14) and showed only marginal activity. Again, the more extensive the degree of alkyl substitution the higher is the dosage required to obtain the optimum effect, although the differences in response are not very distinct [75]. A comparison of the corresponding alkyl and aryl derivatives, 1,2-diaminocyclohexane and *o*-phenylenediamine shows that both are active against the sensitive plasma cell tumour, while both are virtually inactive against S.180.

At present there is no obvious explanation for the variation of activity with amine structure and this requires a great deal of clarification. Interpretation of the

present data is complicated by the fact that some of the compounds appear to be specifically active against the ADJ/PC6A tumour. However, this particularly sensitive system has revealed marked variation in activity for relatively slight changes in amine structure. Kinetic effects alone are unlikely to account for the variations and it has been suggested that hydrogen bonding interactions may be important in stabilising a receptor–drug complex [75]. Certainly increasing alkyl substitution of NH_3 will tend to decrease the H-bonding potential but this does not explain many of the other variations. Determination of relative lipid solubilities are obviously called for (partition coefficients between octanol and water are often used) as membrane interactions are likely to be very important.

Relatively few compounds have been tested in which the amine ligands have been replaced by non-N-donor ligands and none has shown activity [75] (Table 15). This is another area which is worthy of systematic investigation as there is no justification at present to suggest that these complexes cannot give rise to anti-tumour activity, although there does appear to be an empirical preference for amine systems. Apart from oxygen, most of these will involve ligands which are more strongly labilising neutral groups (S- and P-donors and π-acceptor systems) or charged ligands which yield charged complexes.

(iv) Charge effects

Charge type appears to play an important role in the anti-tumour property and as yet only neutral complexes have shown appreciable activity. Even when the criterion of *cis*-leaving groups (of intermediate leaving ability, i.e. chlorides) has been

TABLE 16
Animal testing results for some charged Pt^{II} species (ref. 75)[b]

Complex	Solvent	Dose range (mg/kg)	Dose response	Toxic level (mg/kg)	T/C	Dose (mg/kg)
$K_2[PtCl_4]$	S	10–100	–	40–50	127	25
$K_2[PtBr_4]$	S	3.5–60	±	~ 45	73	15–30
$K_2[Pt(ox)_2] \cdot 2H_2O$	W	5–160	–	~ 40[a]	91	10–40
$K_2[Pt(mal)_2] \cdot 2H_2O$	W	20–250	±	> 250	60	20–250
$K[Pt(C_2H_4)Cl_3]$	S	5–40	–	> 40	122	5–40
$K[Pt(gly)Cl_2]$	S	10–200	–	125–150	79	40–100
$[Pt(NH_3)_4][Pt(NH_3)Cl_3]_2$	S.S.	20–80	+	~ 50	35	40
$K_2[Pt(NO_2)Cl_3]$	S	5–200	–	~ 75	81	50
$[Pt(NH_3)_4]Cl_2$	S	5–200	–	> 200	100	5–200
$[Pt(en)_2]Cl_2$	S	5–40	–	> 40	90	5–40
[Pt(dien)Cl]Cl	S	10–100	–	> 100	71	10–100
[Pt(dien)Br]Br	S	10–80	–	> 80	93	10–80
$K[Pt(o\text{-phen-}5\text{-}SO_3)Cl_2]$	S	5–40	–	> 40	58	5–40

[a]Sporadic toxicity over a wide range. [b]Sarcoma 180 in Swiss white mice.

TABLE 17
Solubilities of "active" complexes (37° C) (ref. 75) [c]

Complex	Solvent	Solubility (± 5%) (g/100 ml)	Approximate therapeutic dose [b] (mg/kg)
cis-[PtA$_2$Cl$_2$]			
A = NH_3	S	0.22	8
$MeNH_2$	S	1.38	12–16
$EtNH_2$	S	0.09	35–40 [a]
Me_2NH	S	0.19	
$HOEtNH_2$	S	> 2.00	(125)
A_2 = en	S	0.08	12–16
1,2-pn	S	0.04	(12)
[Pt(NH$_3$)$_2$X]			
X = mal^{2-}	W	0.04	20–24
$Memal^{2-}$	W	0.25	60
$Etmal^{2-}$	W	0.59	80–90
[Pt(en)X]			
X = mal^{2-}	W	1.00	40–60
$Memal^{2-}$	W	0.50	70–90
$Etmal^{2-}$	W	0.10	(> 100) [a]

[a] Injected as partial slurry. [b] Marginally active compounds in parentheses. [c] Activity shown against Sarcoma 180 in Swiss white mice.

satisfied, the charged complexes tested have been inactive and relatively non-toxic (Table 16) [75]. The rates of Pt^{II} substitution reactions are largely independent of charge [84], so the explanation is presumably biophysical in nature and could be related to membrane transport phenomena and/or to the greater efficiency with which charged molecules (which are generally water-soluble) are eliminated from the body. Certainly more soluble active complexes often require higher doses to be effective. This is clearly seen in the malonate series (malonate, methylmalonate, ethylmalonate (Table 17) [75]. Excretion studies on $[Pt(NH_3)_2(Memal)]$ clearly show that it is removed from the body at a much faster rate than *cis*-$[Pt(NH_3)_2Cl_2]$ but the amount retained after 24 h is very similar [81].

(v) Highly toxic compounds

As indicated in Tables 10 and 12, [Pt(en)ox] (p. 371) and some amine aquo species are highly toxic. The animals often go into convulsions as early as five minutes after injection, after which they convulse periodically but lie dormant between

spasms. The order of toxic levels[75] for aquo species examined so far is $MeNH_2 > NH_3 \simeq en > N,N'\text{-}Me_2en > Me_2NH \gtrsim py$. This presumably reflects the reactivity of the various species; this may be increased by inductive effects and decreased by steric hindrance.

Dwyer et al.[104,105] have reported neuromuscular toxicity for some large inert chelated complexes of Co^{III}, Ru^{II}, Ni^{II}, Fe^{II} and Os^{II}. A comparison test using $[Ru(o\text{-phen})_3](ClO_4)_2$ showed that the symptoms were quite different as the mice injected with the Ru^{II} complex were more quickly affected than the Pt-treated ones, and lost essentially all muscular control[75]. The difference in toxic symptoms is, of course, consistent with the chemistry of the two sets of compounds. The platinum species are relatively reactive while most of Dwyer's complexes are unreactive tris-chelates. The only common feature is that both sets are positively charged and Dwyer only observed the effect with cationic complexes. In vitro studies (on toad reactal muscle) suggested curariform activity, which was supported by the synergistic effect of atropine[105]. During the platinum studies $[Pt^{IV}(en)_3]Cl_4$ was shown to be highly toxic and had peculiar effects on mice[75]. This area of the biological activity of metal complexes has attracted very little attention. More research is highly desirable particularly since platinum compounds may be used clinically, and also because metal complexes represent a potential probe for studying events at neuromuscular junctions.

(vi) Platinum(IV) complexes

It has only recently been confirmed that octahedral complexes can show anti-tumour activity ([$Rh(NH_3)_3Cl_3$], p. 383). Thus there is nothing specific in steric terms about the square-planar stereochemistry of Pt^{II}. However, Pt^{IV} is a special case as in a biological medium it is to be expected that reduction to Pt^{II} will occur. It is most noticeable that the two active complexes *cis*-$[Pt(NH_3)_2Cl_4]$ and $[Pt(en)Cl_4]$ are readily reduced to active Pt^{II} complexes with removal of the axial ligands. Urine studies (e.g. chromatography) on animals injected with one of these compounds might provide more direct evidence for this assumption. It is well known that Pt^{IV} complexes are very inert to ligand substitution unless the latter takes place via an equilibrium amount of Pt^{II}. Relatively few Pt^{IV} compounds appear to have been examined as yet; if one was found to be active while its reduction products were not then this would tend to invalidate this theory. As Thomson et al. have pointed out, some Pt^{IV} complexes could still be better anti-tumour agents because of different cell transport properties[71].

E. OTHER METALS: GENERAL CONSIDERATIONS AND RECENT RESEARCH

The key discovery of Rosenberg and his co-workers should stimulate interest in the possible anti-tumour effects of other coordination complexes, particularly those

of the heavier transition metals. At present relatively little research appears to be in progress and it is hoped that this section might encourage more activity in this field. Using the elementary starting supposition that anti-tumour activity requires a complex of intermediate lability, fundamental coordination chemistry can help to postulate the metal systems which stand a good chance of success. The complex *cis*-$[Pt(NH_3)_2Cl_2]$ differs from most of the agents described in Section B in two major ways: (*a*) it contains simple inorganic ligands which have no carcinostatic ability in their own right and cannot contribute to the anti-tumour effect, (*b*) it contains a heavy third-row transition metal which is not normally found in cells (unlike Cu, Co, Zn) and even in very small amounts it is likely to form lesions in macromolecules (i.e. reaction with functional groups) which the cells are unable to repair. Thus we can study relatively simple transition metal complexes bearing in mind their major kinetic and thermodynamic properties.

(i) General considerations

In general for metal oxidation states with the same d electronic configuration, inertness to substitution increases in the order 1st row < 2nd row < 3rd row for a given set of ligands. This property of transition metals is presumably reflected in the observation that of the essential transition metals found in biological systems, only Mo is not in the first row. It is likely that second and third row metal complexes are too inert to perform metabolic functions. Within a particular row, independent of mechanism, the contribution of crystal field energy to the activation energy predicts that for octahedral complexes, the following order of inertness will generally occur[84]: d^6 (low) > d^3 > d^4 (low) > d^5 (low); d^0, d^1, d^2, d^5 (high spin), d^6 (high spin), d^7 (high spin) are relatively labile systems compared with the above. The first-row metals are likely to be too labile (particularly as they are generally high spin) except for Co^{III} (d^6 (low spin)) and Cr^{III} (d^3). In the second and third rows most of the potentially useful systems lie amongst the platinum group metals, e.g. Rh^{III}, Ru^{II}, Ir^{III}, Pt^{IV} (d^6); Ru^{III} (d^5); Os^{IV} (d^4). Mo^{III}, W^{III} and Re^{IV} (d^3) are relatively unstable and are likely to hydrolyse under physiological conditions. As Thomson et al. have pointed out[71], the biological system acts at a low redox potential and many metals will tend to exist in their lower oxidation states, e.g. Os^{II}, Ru^{II} rather than Os^{III}, Ru^{III}. This may well be the reason for the activity of certain Pt^{IV} complexes. However, introduction of the metal in a higher oxidation state might be advantageous in terms of cell transport ability. Besides Pt^{II}, the d^8 square planar possibilities are Pd^{II}, Au^{III}, Rh^{I} and Ir^{I} (assuming Ni^{II} is too labile). Under physiological conditions Au^{III} is likely to be reduced to Au^{I} (linear or tetrahedral) and Rh^{I} and Ir^{I} will tend to undergo oxidative addition to the Rh^{III} or Ir^{III} state. For comparable compounds the reaction rates of Ni^{II}, Pd^{II} and Pt^{II} are in the approximate ratio $5 \times 10^6 : 10^5 : 1$ respectively[84]. Au^{III} complexes react at a slightly slower rate than those of Pd^{II}.

Regardless of the metal's oxidation state, the ligands will affect the stability and the kinetic properties of the complex. The heavier transition metals are generally class "b" (soft) in character or are intermediate tending to class "b"[106]. Thus they form their most stable bonds with the heavier donor atoms in Groups V, VI and VII of the Periodic Table and generally the following order of decreasing stability applies: S ~ C > I > Br > Cl > N > O > F. This does not imply rapid replacement of ligands in the above sequence but it does mean that those low in the order will not replace those higher in the order when the two ligand concentrations are approximately equal. This is particularly important when considering the possible sites of reaction with biological macromolecules (Section F). It should be pointed out that Co^{III} and Cr^{III} which are potentially useful systems on kinetic grounds, are class "a" (hard) in nature and for these the reverse order will generally apply. The ligands affect the kinetics of the complex by means of their leaving abilities. The order of leaving ability for most Pt^{II} reactions is given on p. 369, and this corresponds to the stability order of the ligand binding to the metal. In reactions where bond breaking is important then the order of bond stability will largely parallel the order of leaving ability; this will particularly apply to octahedral complexes whose reaction mechanisms are generally dissociative. The labilising effect of strongly bound *trans* ligands is especially important in square-planar systems. A more detailed consideration of the kinetic and thermodynamic properties of complexes related to *cis*-$[Pt(NH_3)_2Cl_2]$ is to be found in ref. 71. Williams has recently reviewed some general aspects of metals in relation to cancer[107] and has also considered drug design for amino acid complexes[108].

(ii) Recent research

(a) Palladium

Some Pd^{II} analogues of active Pt^{II} complexes have been tested against S.180 (Table 18)[75], but no significant activity was observed. Some of the compounds were tested on bacteria (*E. coli B*)[109] and although they were bacteriostatic at low con-

TABLE 18
Animal testing results for palladium(II) complexes (ref. 75)[a]

Complex	Solvent	Dose range (mg/kg)	Dose response	Toxic level (mg/kg)	*T/C*	Dose (mg/kg)
cis-$[Pd(NH_3)_2Cl_2]$	S	1.25–10	–	> 10	83	1.25–10
$[Pd(en)Cl_2]$	S.S.	5–40	–	> 40	79	5–40
$[Pd(en)Cl_2]$	S.S.	10–50[b]	–	> 50	78	10–50
$[Pd(NH_3)_2mal]$	W.S.	25–200	±	150–200	55	75–150
[Pd(en)mal]	W	25–100	–	> 100	75	25–100
[Pd(en)ox]	W	1.25–75	–	> 40	104	1.25–25
$[Pd(NH_3)_2ox]$	S.S.	1.25–200	–	~ 100	80	12.5–80

[a]Sarcoma 180 in Swiss white mice. [b]Four injections/day for 2 days.

centrations, no filamentation was induced. $[Pd(dtp)_2]$ (dtp = *O,O'*-dimethyl dithiophosphate) is somewhat active against the Walker 256 carcinosarcoma in mice and reduced the tumours to 6% relative to the controls; no other details are given[110]. It seems reasonable to speculate that Pd^{II} complexes are too reactive in vivo and on this basis complexes of Ni^{II} and Au^{III} are unlikely to be effective unless strongly deactivating ligands are present. However, in this regard the results for the presumably less reactive bis-chelated compounds in Table 18 are not very encouraging[75].

(b) Rhodium

The study of biological activity in rhodium complexes has been pioneered by Gillard and his co-workers[111], who reported on the anti-bacterial activity of a large number of complexes of the types *trans*-$[RhL_4X_2]Y$ and $[Rh(B_2)X_2]$ (L = pyridine or substituted pyridines; B = 2,2'-bipyridyl, 1,10-phenanthroline, ethylenediamine; X = *Cl*, Br; Y = *Cl*, Br, NO_3, ClO_4). Only the complexes of type *trans*-$[RhL_4X_2]Y$ were found to have high levels of antibacterial activity and these complexes were more effective against gram-positive than gram-negative bacteria (except *E. coli*). The active complexes force filamentation at sub-lethal doses indicating that like the Pt complexes they are interfering with cell division. When X = Br in place of Cl, the activity was enhanced (approximately tenfold) but variations in the counterion Y had no effect. In some tests the activity against gram-positive organisms increased with an increasing number of carbon atoms in the alkyl side chain in either the 3, 4 or 5 position of the pyridine ring. This was thought to be due to increased lipid solubility which would give rise to increased cell wall penetration. Pyridine, substituted pyridines and rhodium trichloride did not affect bacterial growth. The complexes $[RhB_2X_2]Y$ were inactive; they had a *cis* configuration. Recent results indicate that a Rh^I species (presumably $[Rh(py)_4]^+$) is involved and that biological activity is closely related to the redox potential for a given complex[112]. It is well known that many reactions of *trans*-$[Rh(py)_4Cl_2]^+$ proceed via an equilibrium concentration of Rh^I. Despite the fact that Pt^{II} and Rh^I are both d^8 systems, the rhodium complexes are *trans* and a different in vivo mechanism could be involved in each case. $[Rh(py)_4X_2]X$ (X = Cl, Br) have been tested for anti-tumour activity against the S.180 system but showed only marginal activity (best *T/C*, 42%; toxic level, 40 mg/kg; X = Cl)[113]. Further testing is desirable, especially as two other rhodium complexes have recently been reported as showing anti-tumour effects. *mer*-$[Rh(NH_3)_3Cl_3]$ was tested on the basis of the criteria obtained from the platinum compounds, namely those of neutrality and *cis* leaving groups. It has shown limited activity against S.180 and good activity against the Walker 256 carcinosarcoma in rats, and is at present undergoing more extensive testing[77,113]. Several other Rh complexes were inactive against S.180 (Table 19)[113]. Rhodium acetate, $[Rh_2(OAc)_4]$, has been used in combination therapy with arabinosylcytosine in the treatment of BDF_1 mice[114] bearing L.1210. Arabinosylcytosine (5–80 mg/kg) and rhodium acetate (1.5–6.25 mg/kg) were given separately every 8 h with 4 h intervals between each

TABLE 19
Anti-tumour activity of rhodium amine complexes

Complex	Solvent	Dose range	Dose response	Toxic level	T/C	Dose
Sarcoma 180[a]						
mer-[$Rh(NH_3)_3Cl_3$]	S.S.	12.5–100	+	70–100	17	100[b]
	S	5–30[c]	+	~20	31	20
mer-[Rh(dien)Cl_3]	S.S.	25–100	±	> 100	54	50–100
trans-[$Rh(NH_3)_3(H_2O)Cl_2$]-NO_3	W	50–150	±	125–150	48	150
[$Rh(NH_3)_3$(ox)Cl]	W.S.	50–125	–	> 125	73	125
trans-[$Rh(NH_3)_4Cl_2$]NO_3	W	25–100	–	> 100	82	25–100
cis-[$Rh(en)_2Cl_2$]NO_3	S	50–200	±	> 200	63	50–200
trans-[$Rh(en)_2Cl_2$]NO_3	S	5–50	±	> 50	46	50
Cs[Rh(en)Cl_4]	S	50–200	–	~200	63	100–200
mer-[$Rh(py)_3Cl_3$]	S.S.	50–200	±	~100	51	100[b]
trans-[$Rh(py)_4Cl_2$]Cl	S	20–50	±	40–50	45	50[b]
Sarcoma 180 (Ascites)[a]						
mer-[$Rh(NH_3)_3Cl_3$]	S.S.	50–100	+	> 100	23%[f] (I.L.S.)	100

	Solvent	Dose range	Dose response	$L.D_{50}$ (mg/kg)	$I.D_{90}$ (mg/kg)	T.I.
ADJ/PC6A[d]						
mer-[$Rh(NH_3)_3Cl_3$]	A	4–500	+	235	86	2.6
mer-[$Rh(NH_3)_3(NO_3)_3$]	A	12–1500	+	135	59	2.3
Walker 256[e] *carcinosarcoma*						
mer-[$Rh(NH_3)_3Cl_3$]	A	5–160	+	~170	~40	~4

[a]Swiss white mice. [b]66% survivors only. [c]Daily dose for 8 days. [d]BALB/$\bar{c}$ mice. [e]Rats. [f]Percentage increase in lifespan (max = ~100%).

drug. The combination was found to act synergistically, giving a 50 day survival rate of 14–100% depending on the number of L.1210 cells implanted and the length of therapy. Mice which received rhodium acetate and survived for 50 days showed no signs indicating delayed toxicity during another 100 days. No data were reported for the rhodium complex alone, but it was found to bind strongly to DNA yielding a blue complex, and it also caused 50% inhibition of the L.1210 DNA polymerase activity at a concentration 0.125 m*M*. Thus the synergistic effect appears to result from a concerted attack on DNA. These recently reported observations indicate that rhodium complexes may be of considerable biological significance.

(c) Iridium

Gale et al.[115] have reported activity against the Ehrlich ascites tumour (BALB/$\bar{c}$ mice) for a violet solution prepared by irradiating with ultraviolet light a solution of

$(NH_4)_2[IrCl_6]$ in ammonia for 7 h. The activity was limited to a maximum 140% increase in lifespan using a daily dose of 100 mg/kg for 8 days. Further testing against L.1210 (BDF_1 mice) indicated a low activity with a maximum increase in lifespan of 20%. No evidence was obtained that this product is a selective inhibitor of DNA synthesis. It is suggested that the irradiated solution contains the Ir^{IV} complex *cis*-$[Ir(NH_3)_2Cl_4]$, purely on the basis of analogy with the $(NH_4)_2[PtCl_6]$ system. However, a polynuclear mixed oxidation state (Ir^{III}, Ir^{IV}) species would seem more likely. This solution does cause filamentous growth in *E. coli* [16]. Non-irradiated solutions were inhibitory to bacterial growth (> 10 μg/ml) but after irradiation for 1 h virtually no inhibition of growth was evident even at 40 μg/ml. After irradiation for 2 h the solutions were still not substantially inhibitory to growth but did induce some filamentation which increased as the radiation was continued for up to 7 h. After irradiation for 24 h the incidence of elongated forms was considerably less than after 7 h. Electrophoresis measurements were consistent with the conversion of a strongly negative species through a less negative species to a final neutral species.

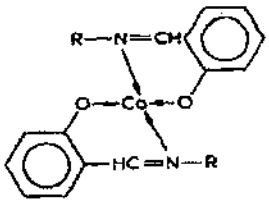
Some Ir^{III} amine complexes have been examined for biological activity against *E. coli* and S.180 (Table 20) but no activity was observed [113]. Ir^{III} complexes are known to be extremely inert and this is likely to be the reason for their inactivity and particularly their lack of toxicity. The presence of Ir^{IV} (d^5) in the irradiated $(NH_4)_2[IrCl_6]$ solution might make it somewhat more reactive.

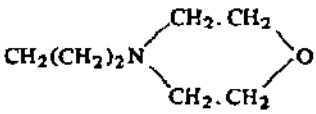
TABLE 20
Anti-tumour screening on iridium amine complexes (ref.113)

Complex	Solvent	Dose range (mg/kg)	Dose response	Toxic level (mg/kg)	T/C	Dose (mg/kg)
Sarcoma 180[a]						
mer-$[Ir(NH_3)_3Cl_3]$	W.S.	50–200	–	150–200	76	200[b]
cis-$[Ir(en)_2Cl_2]NO_3$	S	50–200	–	> 200	94	50–200
trans-$[Ir(en)_2Cl_2]NO_3$	S	50–200	–	> 200	109	50–200
trans-$[Ir(NH_3)_4Cl_2]Cl$	S	50–200	–	> 200	75	50–200
$Cs[Ir(en)Cl_4]$	S	50–200	–	> 200	95	50–200
$Cs[Ir(NH_3)_2Cl_4]$	S	50–200	–	> 200	111	50–200
Sarcoma 180 (Ascites)[a]						
mer-$[Ir(NH_3)_3Cl_3]$	S.S.	125–200	–	> 200	[d]	125–200
	Solvent	Dose range (mg/kg)	Dose response	$L.D_{50}$ (mg/kg)	$I.D_{90}$ (mg/kg)	T.I.
ADJ/PC6A[c]						
mer-$[Ir(NH_3)_3Cl_3]$	A	12–1500	–	> 1500		

[a]Swiss white mice. [b]66% survivors. [c]BALB/c mice. [d]No increase in lifespan.

TABLE 21
Screening results for Co^{II} Schiff base complexes against the Walker tumour in rats



R	Dose range[a,c]	Dose response	Best T/C[b]	Dose
$(CH_2)_2CH_2OH$	9.4–37.5 (3)	+	21	37.5
$C(CH_3)_2CH_2OH$	12.5–100 (4)	+	24	100
$C(CH_3)(CH_2OH)_2$	50–100 (2)	+	56	100
$C(C_2H_5)(CH_2OH_2)_2$	50–200 (3)	+	38	200
$CH_2.CH_2.OC_2H_5$	50–100 (2)	+	43	100
$CH_2.(CH_2)_2.NH(CH_2)_2OH$	25–100 (3)	+	43	100
	12.5–50 (3)	+	29	50

[a]Number of dose levels in parentheses. [b]No indication of toxic levels is given and better results may be obtainable. [c]One dose daily for 4 days; administered i.p. as a suspension in an unspecified medium.

(d) Ruthenium

Certain Ru^{III} complexes cause filamentous growth in *E. coli* (Table 6)[6,67,109]. $[Ru(NH_3)_3Cl_3]$ (probably *mer*) is particularly effective but anti-tumour screening against S.180 was inconclusive[107,113]. However, ruthenium complexes are worthy of more extensive investigation.

(e) Other metals

Schiff-base complexes of Co^{II} (presumably square-planar) have shown limited activity against the Walker sarcoma in rats (Table 21)[117]. Some Schiff bases are known to have anti-tumour activity in their own right and these complexes will be fairly labile to ligand substitution (d^7)[118,119]. Oxidation to Co^{III} could also be important. It is claimed[110] that $[Ni(dtp)_2]$ (dtp = dimethyl dithiophosphate) is also active against the Walker system but no data are given, and the compound appears to be inactive against L.1210.

F. INTERACTION OF METAL COMPLEXES WITH BIOLOGICAL MOLECULES: MECHANISM OF ANTI-TUMOUR ACTION

The interaction of coordination complexes with biological metabolites and

macromolecules is a subject capable of filling several reviews in its own right. Thomson et al. have discussed these reactions in general terms for platinum compounds[71], while Izatt et al. have extensively reviewed metal interactions with nucleic acids and their constituents[120]. Here it is intended briefly to discuss the evidence for the likely sites of action of the established platinum drugs and to consider the type of studies which are required to elucidate more information.

(i) Evidence for interaction with nuclear DNA

Two major studies on the incorporation in vitro of the labelled precursors of DNA (thymidine-^{3}H) RNA (uridine-^{3}H) and protein (L-leucine-^{3}H) in the presence of *cis*-$[Pt(NH_3)_2Cl_2]$, agree on the finding that the drug selectively inhibits DNA synthesis at low concentrations. Harder and Rosenberg have studied this incorporation into human amnion AV_3 cells and find selective inhibition of DNA synthesis using the platinum solution at 5 μM and less[121] (Fig. 6). At high concentrations DNA synthesis was more rapidly inhibited than RNA and protein synthesis although after 24 h of treatment all three were virtually completely inhibited. They also compared these effects for four other platinum compounds, two of which were anti-tumour active (*cis*-$[Pt(NH_3)_2Cl_4]$, $[Pt(en)Cl_2]$) and two were inactive ($[Pt(NH_3)_4]Cl_2$, *trans*-$[Pt(NH_3)_2Cl_4]$). As expected the inactive compounds showed little inhibition of DNA synthesis at concentrations comparable to those at which the active compounds were inhibitory (Fig. 7). Gale and Howle[122] studied the incorporation of labelled precursors into Ehrlich ascites tumour cells, which were periodically re-

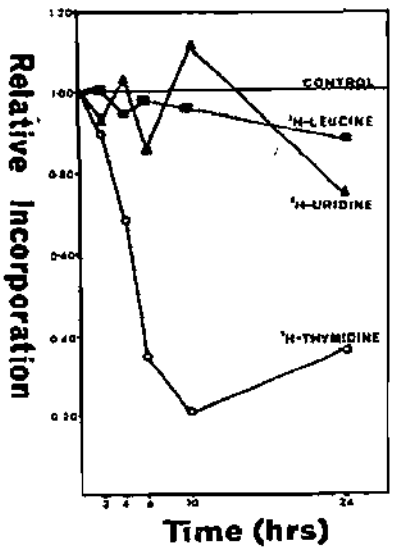


Fig. 6. The selective inhibition of DNA synthesis in human AV_3 cells grown in tissue culture with exposure to 5 μM *cis*-$[Pt(NH_3)_2Cl_2]$ (ref. 72, 129).

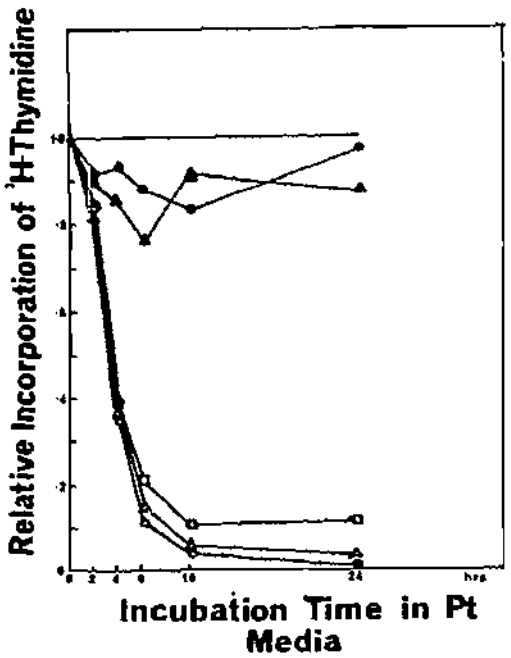


Fig. 7. The effect of various platinum complexes on the rate of DNA synthesis in human AV_3 cells in culture. The anti-tumour active compounds cause a marked inhibition (ref. 121). (●), 25 μM $[Pt(NH_3)_4]Cl_2$; (▲), 25 μM *trans*-$[Pt(NH_3)_2Cl_4]$; (□), 25 μM $[Pt(en)Cl_2]$; (△), 25 μM *cis*-$[Pt(NH_3)_2Cl_4]$; (○), *cis*-$[Pt(NH_3)_2$-.

moved from rats up to 4 days after treatment with a single injection of 10 mg/kg of *cis*-$[Pt(NH_3)_2Cl_2]$. The pattern of subsequent synthesis of DNA, RNA and protein was followed in vitro (Fig. 8). Initially there was a marked impairment for all three labelled precursors but subsequently the rates of uridine (RNA) and L-leucine (protein) returned to control values, while a striking suppression of the rate of thymidine incorporation persisted for at least 96 h. When Ehrlich ascites tumour cells were treated in vitro with *cis*-$[Pt(NH_3)_2Cl_2]$ (100 μ*M*), DNA, RNA and protein synthesis were inhibited in that order, in agreement with the results of Harder and Rosenberg. *cis*-$[Pt(NH_3)_2Cl_2]$ is similarly a potent and probably irreversible inhib-

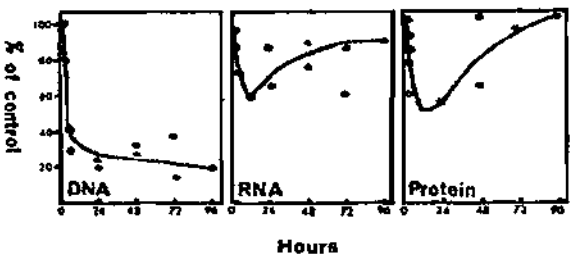


Fig. 8. The pattern of DNA, RNA and protein synthesis in Ehrlich ascites tumour cells extracted from mice treated with *cis*-$[Pt(NH_3)_2Cl_2]$. There is long-term suppression (> 96 h) of DNA synthesis (ref. 122).

itor of DNA synthesis in cultures of human lympocytes under mitogenic stimulation (phytohaemagglutinin-P)[123]. At low, pharmacologically realistic concentrations ($\sim 3\ \mu M$) the selectivity was quite evident, with RNA and protein synthesis being only slightly diminished. Gale and Howle also carried out similar studies on *cis*-$[Pt(py)_2Cl_2]$ which had shown some activity against the Ehrlich ascites tumour in mice[102]. A moderate degree of selectivity against DNA synthesis was observed. This was significantly less than found for the ammine complex, which correlates with the lowered anti-tumour activity. Studies on a tritiated sample of the pyridine complex confirmed strong bonding to various nucleic acids with a possible preference for guanylate and uridylate sites[103]. It would appear from these results that the primary mechanism of action of the platinum drugs resides in their ability selectively to inhibit DNA synthesis. The relatively slow response of DNA synthesis to the platinum compounds led Gale and Howle to suggest a two-stage transformation[123] $A \rightarrow B \rightarrow C$, where A is *cis*-$[Pt(NH_3)_2Cl_2]$ and B is non-selective against DNA, RNA or protein synthesis (suggested to be the $Pt(NH_3)_2Cl^+$ moiety) while C is a slowly formed agent which is selective against DNA synthesis, e.g. $(Pt(NH_3)_2^{2+})$. Rosenberg and Harder suggest $A \rightarrow B \rightleftharpoons C$, where B is a slowly increasing species to which DNA synthesis is selectively sensitive, while C is non-selective[121]. From a chemical viewpoint these arguments appear invalid, as all reactive platinum species would interact as readily with the nucleophilic centres in RNA and particularly protein (especially at sulphur sites) as with DNA. Platinum(II) substitution reactions all proceed to some extent by a solvent-assisted path[64]. Two of the many factors which are likely to be important in this slow onset of inhibition of DNA synthesis are (*a*) the rate of transport through the cell-membrane, and (*b*) the variations of chloride concentration which will inhibit reactions of *cis*-$[Pt(NH_3)_2Cl_2]$. The high chloride concentration in the serum will at least partially protect the complex prior to entering the cell. The concentration in the cytoplasm is still considerable and will enforce a slow rate of reaction. The concentration within the nucleus is unknown, but if it is low it will allow a degree of preferential reaction with DNA. Williams has suggested that the ordered structure of DNA with its parallel planar bases may allow the planar Pt^{II} complexes to form a strong association prior to attack[124] (the activity of octahedral complexes may tend to invalidate this), although this argument should also apply to RNA. The nucleic acid structure is more amenable to chelation binding than the random distribution of side chains in proteins, and in any case the hydrogen-bonded structure of the nucleic acid may be more easily perturbed. It is appreciated that the metal will also react with RNA and particularly with proteins; secondary effects may be due to these interactions. Indeed it is most unlikely that all actions of metal drugs will be at the DNA level.

The ability of the platinum compounds to attack DNA suggests a similarity to the bifunctional alkylating agents (e.g. nitrogen mustard, Fig. 9), which are also broad-range anti-tumour agents[3,4]. These are thought to exert their biological activ-

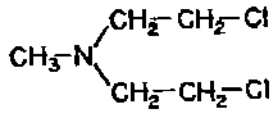
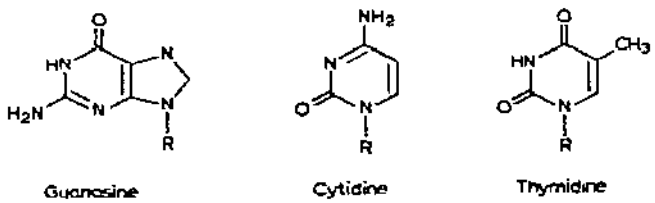


Fig. 9. Nitrogen mustard (HN-2) or methyl bis(2-chloroethyl)amine. Dispensed as the hydrochloride.

ity by cross-linking opposite strands of DNA via the N-7 positions of guanine[125] (Fig. 10). However, the leaving groups in bifunctional alkylating agents have a maximum separation of 8 Å while the chlorides in *cis*-$[Pt(NH_3)_2Cl_2]$ are some 3.3 Å apart[126]. Nevertheless, Roberts and Pascoe have demonstrated[127] the formation of interstrand cross-links in DNA from HeLa cells in tissue culture treated with *cis*-$[Pt(NH_3)_2Cl_2]$, using a method developed to demonstrate unequivocally the formation of interstrand DNA links in cells treated with the bifunctional alkylating agent mustard gas (Fig. 11)[128]. Cells were grown for one generation (24 h) in the presence of 5-bromo-2'-deoxyuridine (BUdr) to give one strand of their DNA a density label. When the DNA of drug treated cells was subjected to alkaline caesium chloride gradient centrifugation, heavy, light and hybrid strands were separated. The hybrid represented interstrand cross-linking between light and heavy DNA and was found for both mustard gas and *cis*-$[Pt(NH_3)_2Cl_2]$. Further experiments using a radioactively labelled heavy DNA (^{3}H-deoxythymidine (TdR) + BUdr) indicated



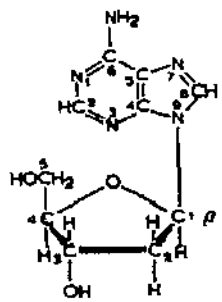


Fig. 10. The major DNA nucleosides. All nucleosides mentioned in the text contain deoxyribose (R) as shown for adenosine.

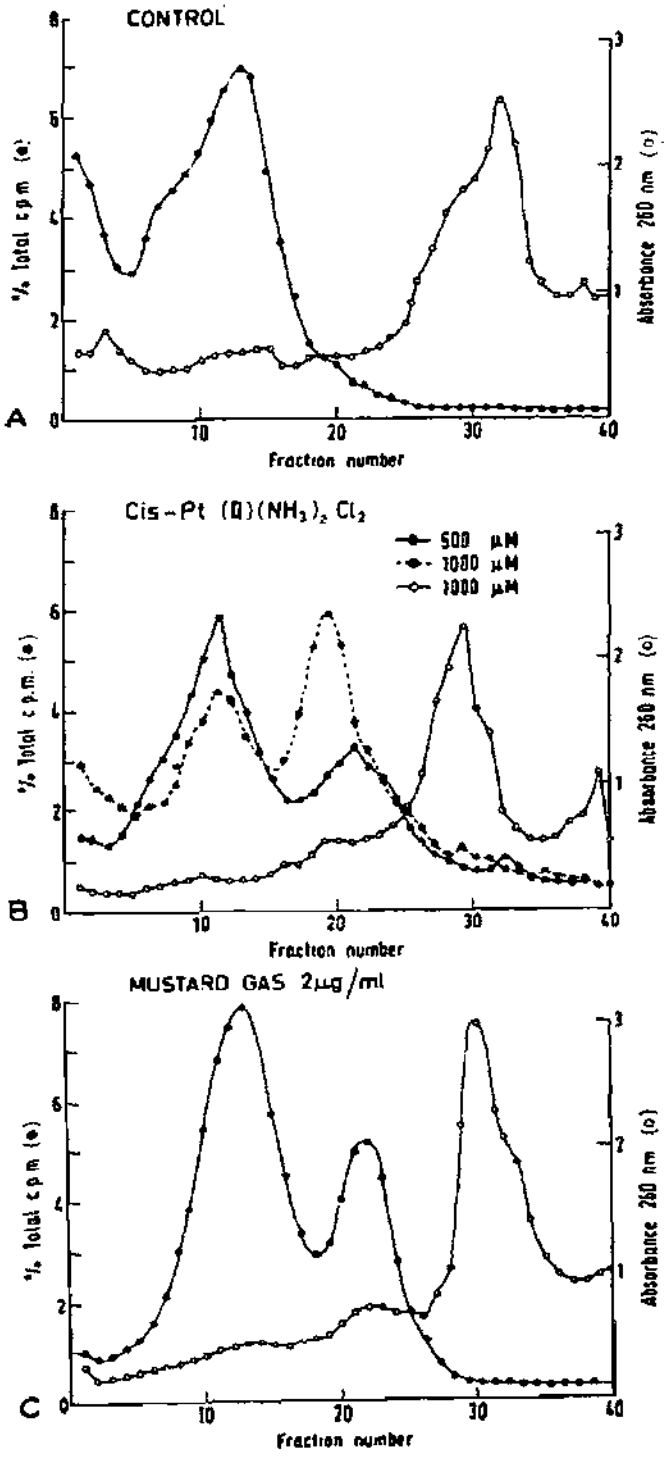


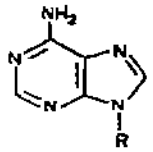
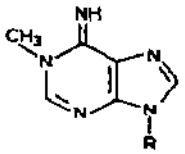
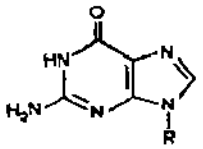
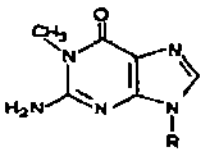
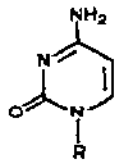
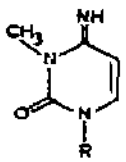
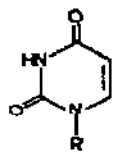
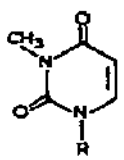
Fig. 11. Detection of cross-linked DNA in HeLa cells treated with *cis*-$[Pt(NH_3)_2Cl_2]$ and mustard gas. Fractions of isolated DNA were taken from a CsCl gradient and studied using absorption at 260 nm and radioactivity counting (% of total counts on the gradient) (ref. 127). A. Control (●) counts/min; (○) absorbance, 260 nm. B. *cis*-$[Pt(NH_3)_2Cl_2]$; (●—●) c.p.m. 500 μM; (● .... ●) c.p.m. 1000 μM; (○—○) absorbance, 260 nm, 1000 μM. C. Nitrogen mustard (2 μg/ml); (●) c.p.m.; (○) absorbance, 260 nm.

that at a platinum complex concentration of 3 μM approximately 0.2% of the DNA is cross-linked. The percentage cross-linking of heavy and light strands in a given situation depends on the molecular weight of the DNA isolated. Thus it was estimated, by comparing the molecular weight of the DNA isolated with known estimates of that in the nucleus, that the true amount of cross-linking is approximately 2%. This compares with a corrected value of 10% for mustard gas at a comparable dose[128] (i.e. the maximum dose at which all cells survive, D_0). The possibility of platinum cross-linking via nucleophilic attack on the nucleoprotein coat of the DNA was eliminated by isolating DNA from cells which had been given the same radioactive and density labels, and treating this with various concentrations of the drug. Cross-linking was still observed under these conditions and could not have involved proteins. Thus one of the in vivo reactions of *cis*-$[Pt(NH_3)_2Cl_2]$ is to cross-link DNA and this has also been demonstrated for $[Pt(en)Cl_2]$. Suitable sites must be some 3Å apart and it has been suggested that the 6-amino groups of adenine in an ApT sequence, the 2-amino groups of guanine in the narrow groove or the 6-amino groups of cytosine in the wide groove of a CpG sequence, are possible sites especially as the amino groups are generally well exposed to attack by external agents[127]. As Harder has suggested, intrastrand links are also possible[129], and it has been proposed that these might be largely responsible for the ability of Pt compounds to inactivate bacteriophage[130]. Small amounts of *cis*- and *trans*-$[Pt(NH_3)_2Cl_2]$ and $[Pt(en)Cl_2]$ were found to inactivate T7, a double-stranded DNA phage (0.007–0.5 μg/ml), and R17, a single-stranded RNA phage (0.09–1.1 μg/ml). In both cases the toxicity decreased in the order $[Pt(en)Cl_2] >$ *cis*-$[Pt(NH_3)_2Cl_2] \gg$ *trans*-$[Pt(NH_3)_2Cl_2]$. Using $[Pt(^{14}C\text{-}en)Cl_2]$ it was found that 1.5 and 5 molecules of the Pt complex were bound to R17 and T7 respectively at the mean lethal doses; 96% of the Pt bound to R17 was on the RNA and 76% of the Pt bound to T7 was on the DNA. Studies on DNA isolated from Pt-treated T7 showed that all three complexes could bind in a manner involving cross-linking of two DNA chains. The amount of cross-linking was small although $[Pt(en)Cl_2]$ and *cis*-$[Pt(NH_3)_2Cl_2]$ were again comparable while *trans*-$[Pt(NH_3)_2Cl_2]$ was less effective. The mean concentrations for cross-linking were in general much higher than the estimates of the mean lethal dose, indicating that this reaction was not a major contribution to the inactivation. In the $[Pt(^{14}C\text{-}en)Cl_2]$ case only one thirtieth of the bound molecules formed interstrand links. Linking of nucleic acid to protein was detected but was thought to be an infrequent reaction. Analysis of the products of reaction of mustard gas with similar bacteriophages suggests that intrastrand linking is the important inactivating reaction[131,132], and it is likely that this also applies to the Pt compounds. These authors note the tendency of $[Pt(en)Cl_2]$ and *cis*-$[Pt(NH_3)_2Cl_2]$ to form blue solutions on long standing in the phosphate buffer medium. These polymerisation effects are presumably similar to those found in sulphate media and are likely to involve metal–metal interactions[133]; they are unlikely to be of significance in vivo.

(ii) Interactions with DNA and its components in vitro

It has been established that the likely mode of anti-tumour action of Pt compounds is by producing a primary lesion in DNA; several studies on the nature of this interaction have been reported. Mansy et al. have studied the binding of both *cis*- and *trans*-$[Pt(NH_3)_2Cl_2]$ to simple nucleosides (Fig. 11) and following this to oligomers and polymers of nucleic acid bases[134–136]. Since the Pt complexes have only weak absorptions in the ultraviolet (210–270 nm), they used differential spectrophotometry to follow the reactions. In this manner binding of metal ions to sites on the aromatic purine and pyrimidine rings is observed but no information can be obtained about possible binding to the sugar or phosphate residues. Several techniques were used to determine the possible sites of attack. Firstly the pH dependence of the Pt binding was studied and literature pK_a values of the ring nitrogen atoms used to predict the extent of binding. Secondly a check of these conclusions was obtained by observing the binding to nucleosides with successive positions blocked by methyl groups. Also as only the *cis* compound can bind to two sites on one base, a comparison of the interactions enabled a distinction to be made between bidentate and monodentate binding. The possible complication due to bridging between nucleosides was minimised by working at Pt-to-ligand ratios greater than 1.0. The reactions were carried out in aqueous 0.1 *M* $NaClO_4$ at 37°C and as several days were often required before equilibrium was established, the reacting species were hydrolysis products of the chloroammine compounds. The complexes did not react in the unaquated form. The results demonstrate that both *cis*- and *trans*-$[Pt(NH_3)_2Cl_2]$ readily bind to the ring nitrogens of the purines and pyrimidines and the sites occupied are generally those favourable to proton binding (Table 22). The *cis* isomer forms a bidentate chelate with either 6-NH_2 + N-7 or 6-NH_2 + N-1 of adenosine and 4-NH_2 + N-3 of cytidine. The *trans* isomer interacts monofunctionally at N-7, N-1 of adenosine and N-3 of cytidine, while both isomers bind monofunctionally to N-7 of guanosine and inosine. Formation constants for guanosine ($\sim 10^9$) were much higher than for adenosine ($\sim 10^4$) and cytidine ($\sim 10^5$). No binding to the ring nitrogen of uridine or thymidine or monofunctional attack at the NH_2 groups of cytidine, adenosine or guanosine was detected. The authors suggest that the amino groups of adenosine and cytidine will be occupied when they are sterically well disposed for bidentate chelation, as is the case in DNA. Robins studied the reactions of nucleosides but used $[Pt(^{14}C\text{-en})Cl_2]$ (apparently mixed with the Magnus-type salt $[Pt(en)_2][PtCl_4]$) with chromatographic separation of the reaction mixture[137,138]. Guanosine and ATP reacted more rapidly than adenosine or cytidine, and thymine derivatives showed no reaction (in agreement with the findings of Mansy et al.[135]). Chromatographically distinct products were formed with ^{3}H-adenine, ^{3}H-adenosine, ^{3}H-guanine and ^{3}H-guanosine and the Pt-to-base ratio was very close to unity. There was evidence for a second 2 : 1 product with ATP which may involve phosphate binding (see p. 398). Thomson and Mansy examined the possibility of cross-linking via the 6-amino groups

TABLE 22
Binding of purines and pyrimidines to platinum complexes

	pH	N_1	N_7	$-NH_2-$ chelation	Type of complex	Structural formula
Purines						
Adenosine						
cis	5.6	+	+	+	PtL, Pt_2L	
trans	5.6	+	+	–	PtL, Pt_2L	
1-Me-Adenosine						
cis	3.27	–	+	+	PtL	
trans	3.27	–	+	–	PtL	
Guanosine						
cis	5.6	–	+	–	PtL_2	
trans	5.6	–	+	–	PtL_2	
1-Me-Guanosine						
cis	5.6	–	+	–	PtL_2	
trans	5.6	–	+	–	PtL_2	
Pyrimidines						
Cytidine						
cis	5.6	+		+	PtL	
trans	5.6	+		–	PtL_2	
1-Me-Cytidine						
cis	3.27	–		–		
trans	3.27	–		–		
Uridine						
cis	5.6	±[a]				
trans	5.6	–				
1-Me-Uridine						
cis	5.6	–		–		
trans	5.6	–		–		

[a]Very weak

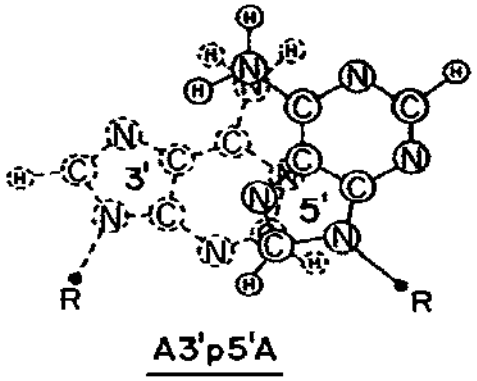


Fig. 12. Structure of diadenosine monophosphate, A3'p5'A (ref. 134).

of adjacent adenosines using the dinucleoside monophosphate, adenosine-3'p5'-adenosine (ApA), which adopts a stacked conformation in solution with the amino groups some 3–4 Å apart [136] (Fig. 12). This stacking gives a characteristic circular dichroism spectrum which decreases as the solution is warmed and the dimer unstacks (Fig. 13A). It was shown that after equilibration with an equimolar quantity of *cis*-$[Pt(NH_3)_2Cl_2]$, the circular dichroism signal was independent of temperature indicating that the stacked structure was indeed stabilised by cross-linking (Fig. 13B). In contrast the *trans* isomer causes the dimer to unstack, presumably due to monofunctional attack placing a positive charge on the ring (Fig. 13C). The possibility of cross-links from different dimers was partially eliminated by studies on A3'p5'U*, where no evidence for a stacked dimer was found over a range of mole ratios of Pt to dimer. Polynucleotides denature at acid or alkaline pH, but around pH 5 most of them, including DNA, gave a white precipitate with *cis*- and *trans*-$[Pt(NH_3)_2Cl_2]$ in the concentration range above 1 : 2 of Pt isomer to phosphate. Polyuridylic acid was an exception and no precipitate was formed but on standing a deep blue colour developed (see Addendum). The UV spectra were similar to those of the nucleosides except that no evidence was found for bifunctional binding of the *cis* complex to adenosine.

Horacek and Drobnik [139] have studied the rate of the reaction between *cis*-$[Pt(NH_3)_2Cl_2]$ and calf thymus DNA. They report a shift in the UV absorption maximum from 259 nm to 264 nm. They estimate an average 0.4 binding sites per nucleotide and found that the presence of *cis*-Pt^{II} aided the renaturation of DNA during heating curve experiments. The optimum ratio of Pt/P was 0.05 and at this ratio no UV shift was observed. This can be interpreted as further evidence for cross-linking of DNA strands. Similar work on the individual bases leads these workers also to postulate purine binding.

* Adenosine-3'p5'-uridine (A3'p5'U).

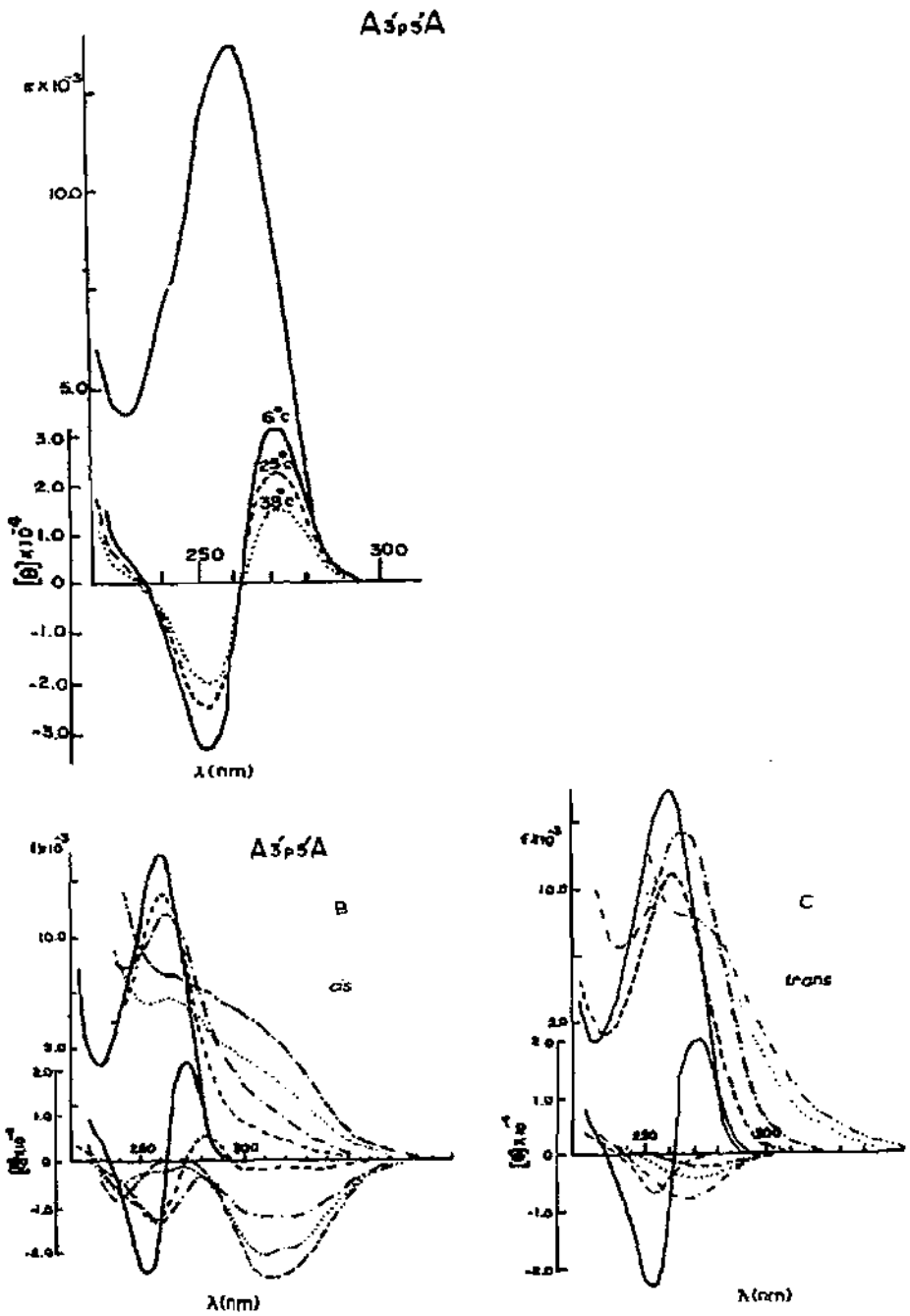


Fig. 13. Absorption and circular dichroism spectrum of adenosine-3'p5'-adenosine (refs. 134, 136). A. A3'p5'A (1×10^{-4} *M*) in 0.1 *M* $NaClO_4$ at 6–38°C. B. A3'p5'A (1×10^{-4} *M*) in equilibrium with *cis*-$[Pt(NH_3)_2Cl_2]$ in 0.1 *M* $NaClO_4$ at 25°C. C. A3'p5'A (1×10^{-4} *M*) in equilibrium with *trans*-$[Pt(NH_3)_2Cl_2]$ in 0.1 *M* $NaClO_4$ at 25°C. For B and C, mole ratios of dimer : Pt; (——) 1 : 0, (——) 1 : 1, (– . – . –) 1 : 3, (. . .) 1 : 5, (– .. – .. –) 1 : 10.

DNA reacts[137,138] more quickly than the simple polymers with both $[Pt(NH_3)_2Cl_2]$ isomers and $[Pt(^{14}C\text{-en})Cl_2]$. The rate of reaction increased for DNA's of increasing GC content. In most cases the *trans* isomer reacted at a slightly faster rate than the *cis*. A break in the line describing the first-order kinetics suggested the possibility of two consecutive mechanisms. It has been tentatively suggested that the initial attack on DNA is at the N-7 of guanine[134,138]. This interaction should be followed by partial denaturation when N-1 atoms of cytidine, adenine and guanine are available. Inter- and intrastrand links are possible.

Studies of this type are obviously at an early stage, but are of great interest to chemists. Relatively few defined compounds have been prepared from transition metals and nucleic acid constituents, particularly among the platinum group metals. The ability of adenosine to chelate has been confirmed by the preparation of a 1 : 1 complex from $K_2[PtCl_4]$ and adenosine[75]; guanosine forms a 2 : 1 compound[140].

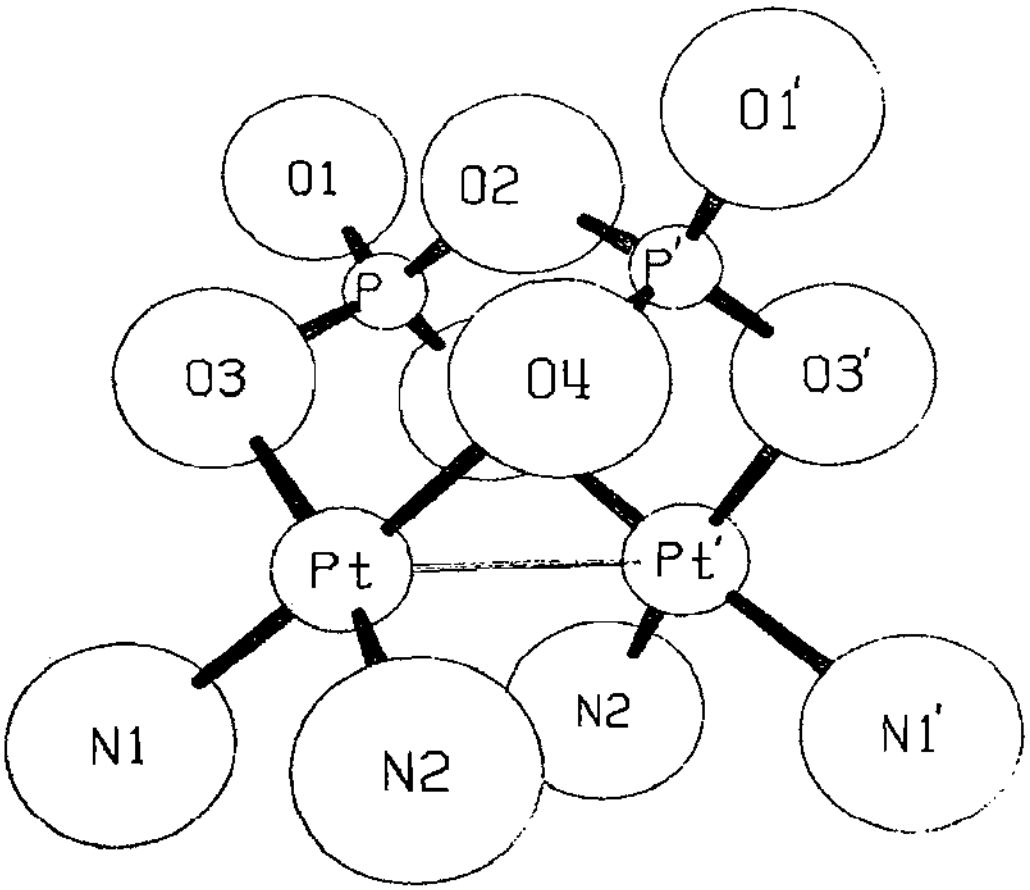


Fig. 14. Structure of pyrophosphatotetramminediplatinum(II), $[\{Pt(NH_3)_2\}_2P_2O_7]$ (ref. 143).

The *trans* derivative $K_2[Pt(adenosine)_2Cl_2]$ has been characterised and is apparently made by the action of excess adenosine on $K_2[PtCl_4]$, although insufficient experimental detail is given[141,142]. The possibility of phosphate binding (p. 393) should not be ignored. Class "a" metals such as Cr^{III}, Mn^{II}, Fe^{II}, Fe^{III}, Co^{II} show a considerable tendency to bind to the phosphate groups[120] and although Pt^{II} would be expected to bind more strongly to the nitrogen atoms in the bases, recent studies have shown the formation of relatively stable pyrophosphatoamine complexes[75]. These are of the type $[(PtA_2)_2P_2O_7]$ and have been shown to contain a bridging pyrophosphate ligand (A = NH_3; Fig. 14)[143]. Thus the binding of the *cis*-(PtA_2) residue to pyrophosphate is stereochemically favourable, and this basic phosphate structure occurs in many biochemically important molecules such as ADP, ATP and other nucleoside di- and triphosphates. Nucleic acids, however, only contain a monophosphodiester bond and the stability of the pyrophosphate complexes probably relies on the bridging ability of pyrophosphate. Studies of the reactions of the various amine complexes described in this review with nucleosides and polymers may be helpful in understanding variations in anti-tumour activity. The preparation of characterisable compounds as well as solution studies, using various spectroscopic techniques, are desirable. Indeed there is some speculation that the combination of a known anti-tumour drug with DNA (or possibly nucleosides or polynucleosides) can lead to enhanced activity[144]. A complex of this type will not diffuse across the cell membrane and requires pinocytosis for uptake followed by lysozyme digestion to release the anti-tumour active portion. The capacity of the compound to kill a particular cell will therefore depend upon the pinocytic, lysomal and mitotic activity of the target cell. Such activity is known to be high in many tumour cells and could form the basis for increased selectivity. This has been successfully applied to daunorubicin, an anti-leukaemia drug[145].

G. CONCLUSION

cis-$[Pt(NH_3)_2Cl_2]$ has just started Phase II clinical trials in the U.S. and it is likely to be several years before the efficacy of platinum drugs against human cancers will be fully known. However, it is clear that cytotoxic drugs like these are not likely to be the panacea for all cancers, although they may be particularly effective in certain areas. If toxic effects remain manageable, they may be a useful addition to the list of cancer chemotherapeutic drugs. The broad spectrum of activity against animal tumours is particularly impressive. Recent results suggest that Pt drugs may be used most effectively in combination with known anti-cancer drugs such as alkylating agents and antimetabolites[146–148]. Several instances of synergistic action have been reported and this type of therapy is also going into human clinical trials. The synergism between Pt complexes and alkylating agents, which are thought to cross-link DNA, suggests a somewhat different mechanism for Pt drugs, possibly due to a greater number of intrastrand links.

Variation of the original structure has shown that activity is found in a variety of compounds and certain empirical rules have emerged, such as the need for neutrality and *cis*-leaving groups of intermediate lability. On the amine side of the molecule alicyclic amines, particularly cyclopentylamine, look particularly promising. At present chloride is the best anionic ligand, but chelating carboxylates are an interesting alternative and form complexes worthy of general study. Application of the ideas generated by the Pt compounds to other metal systems has already produced evidence of anti-tumour activity in Rh^{III} compounds, confirming earlier bacterial work. There is much scope for further research in this area.

Mechanistic studies confirm a selective attack on DNA synthesis although protein and RNA production is affected at higher doses. The initial lesion in DNA is likely to be via the N-7 atom of guanine but although interstrand links have been demonstrated in vivo their location in the structure is not known. Studies in this area are particularly desirable especially the application of such physico-chemical techniques as NMR and vibrational spectroscopy, even though the spectra obtained will be complex. The reactions of other metals with DNA and its constituents may provide an insight into the mechanisms of anti-tumour activity, when carried out in conjunction with an anti-tumour screening programme. Metals provide a valuable and readily detectable probe and therein may lie their greatest contribution, i.e. towards an understanding of the true nature of the disease.

This new class of anti-cancer agent enables coordination chemists to enter the field of drug design. This review has attempted to outline this rapidly developing area of reasearch, and to indicate the ways in which basic coordination chemistry can be applied in the search for better metal-based drugs.

ADDENDUM – SECOND INTERNATIONAL SYMPOSIUM ON PLATINUM COMPLEXES IN CANCER CHEMOTHERAPY

This Symposium was held at Wadham College, Oxford, in April 1973 and included four half-day sessions on the chemistry of the platinum compounds, their reactions with biomacromolecules and their biological effects in in vitro systems and in whole animals. In addition one day was devoted to the results of preliminary clinical trials.

The most interesting chemical discussions centred around new blue platinum complexes containing uracil and thymine ligands. The discovery of this family of compounds was made by Mansy and Rosenberg[149], who prepared a blue complex from a hydrolysed *cis*-$[Pt(NH_3)_2Cl_2]$ solution and uracil. Subsequent screening tests (S.180 ascites) gave good results while the compound had the advantage of high water solubility. The structure and indeed formulation of this compound is as yet undetermined. Although a number of blue platinum compounds have been known for some time, most of them appear to be polymeric with short Pt—Pt distances. One group, however, appears to be monomeric and these have acetamide or substi-

tuted acetamide ligands. The first complex was prepared by Hoffmann and Bugge[158] by hydrolysis of dichlorobis(acetonitrile) platinum(II) with silver sulphate in water to give a blue monomeric acetamide complex which was originally formulated as $[Pt(CH_3CONH_2)_2] \cdot H_2O$. The structure of these amides is still rather uncertain; the latest formulation[156] is $[Pt^{IV}(\text{amide anion})_2(OH)_2]$ (or the corresponding chloride). However, recent ESCA studies[157] indicate the presence of Pt^{II}. The pyrimidine complexes are likely to have a similar structure; the obvious similarity between the amide and pyrimidine ligands lies in the presence of the $-N-\overset{\overset{O}{\|}}{C}-$ grouping. If crystals of $[Pt(CH_3CN)_2Cl_2]$ are left to stand in solutions of 1-methylthymine, 1-methylcytosine or uridine, a blue solution forms within a few hours and this occurs more quickly with DNA. 9-Methyladenine and 9-methylguanine do not give blue solutions so that the colour is unlikely to be due to hydrolysis of acetonitrile[150]. Similarly, *cis*-$[Pt(\text{cyclopropylamine})_2Cl_2]$ gives a purple complex on standing with 1-methylthymine[150]. This compound and several similar "platinum blues" give good screening results. Physical measurements are underway in an attempt to characterise these intriguing compounds.

Other papers discussed the bacterial and anti-tumour activity of rhodium compounds[151] as mentioned in Section E. Preliminary anti-tumour, anti-viral, anti-bacterial and antifungal screenings of some palladium chloro, chloroamine and amino acid complexes were not very encouraging except for *cis*-$[Pd^{IV}(NH_3)_2Cl_4]$, which shows some anti-tumour activity[152].

The molecular structure of the pyrophosphate compound $[\{Pt(NH_3)_2\}_2P_2O_7]$ was reported[153]. Complexes of the type *cis*-$[Pt(am)_2Cl_2]$ (am = $C_nH_{2n-1}NH_2$ with n = 3 to 8) react with DMSO to give *cis*-$[Pt(am)_2Cl(DMSO)]$ (ref. 154). In the presence of chloride the strong *trans*-labilising effect of DMSO sets up the equilibrium *cis*-$[Pt(am)_2(DMSO)Cl]^+ + Cl^- \rightleftharpoons$ *cis*-$[Pt(am)(DMSO)Cl_2]$ + am. The kinetics of the forward reaction have been studied and the rate is first-order with respect to chloride, with the nucleophile-independent pathway (k_1) generally absent. There is no correlation with the potency ($I.D_{90}$) or toxicity ($L.D_{50}$) of the chloroamines. This tends to eliminate the possibility that replacement of the chloride ligands within the body by strong *trans*-labilising groups (e.g. sulphur-containing proteins) followed by labilisation of an amine could provide an alternative mechanistic pathway.

Compounds of the type *cis*-$[PtA_2(nucl)_2]$ (A_2 = en, $(py)_2$ $(NH_3)_2$; nucl = guanosine, inosine) have been prepared and characterised[155]. NMR studies suggest coordination at the N-7 nitrogen.

Most of the clinical papers dealt with phase I studies on *cis*-$[Pt(NH_3)_2Cl_2]$ and were, therefore, accounts of toxicity in man. These studies confirmed the toxicity predicted from dogs and monkeys, of bone marrow depletion and damage to the intestinal mucosa and kidney but not the predicted liver damage. The limiting toxicity is that on the kidney and this is causing the clinicians some concern, although it was pointed out, however, that kidney toxicity need not prevent the use of a drug

if it proved to be highly effective. Kidney toxicity can be minimised by hydration of the patient prior to, and during, infusion of *cis*-Pt^{II}*. Some clinicians reported occasional good responses in the course of the phase I studies particularly with squamous cell carcinomas, ovarian adenocarcinomas and testicular tumours. The general opinion was that although the kidney toxicity of this compound might prove limiting and certainly warranted caution, it was a compound with interesting anti-cancer properties. The clinicians looked forward to the development and availability for clinical trial of the new derivatives reported on during the symposium, all of which are discussed earlier in this review. Such compounds might retain the useful anti-tumour properties of *cis*-$[Pt(NH_3)_2Cl_2]$, but not have the unwanted kidney-damaging properties.

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CONTRIBUTIONS TO THE SECOND INTERNATIONAL CONFERENCE ON PLATINUM COMPLEXES IN CANCER CHEMOTHERAPY

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150 A.J. Thomson and I.A.G. Ross.
151 R.D. Gillard, I.H. Mather and K. Harrison.
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