Recent Results in Cancer Research

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Platinum Coordination Complexes in Cancer Chemotherapy

Edited by T. A. Connors and J. J. Roberts

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With 93 Figures

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Foreword

It was a great pleasure and honour to have been invited to attend this Seminar and to present a final impression. The association in this field between the Chester Beatty Research Institute and Dr. Rosenberg's School at East Lansing is something which I specially value and many would doubtless like to know how it came about.

In the course of its work in carcinogenesis and on chemotherapy over many years, the Chester Beatty Research Institute was frequently drawn to the importance of many metals — as for example lead, iron, metalloid arsenic and the metalloid qualities of the carcinogenic hydrocarbons. Interest started in platinum many years ago, following the possibility, claimed by others, that various complexes between the metal and mercaptopurine might possess significant chemotherapeutic properties. Various attempts to confirm such findings ended, however, in complete failure. Interest in platinum was revived by the fresh observations of Dr. Rosenberg and his colleagues, and here the outcome was entirely different. Very soon it was possible to confirm the intense growth-inhibitory properties of *cis*-platinum (II) diamminedichloride and related substances. After communicating these results to Dr. Rosenberg, it was a pleasure to welcome him in London where he gave a Seminar which greatly engaged the interest of many of the staff. Later, several of these were to enjoy Dr. Rosenberg's hospitality at an international conference on the subject to be held in East Lansing, where many rapidly developing aspects were open for discussion. Thereafter, co-operation between the two groups remained active.

Having some interest in the methods and philosophy of research as well as in its details, the manner of discovery of the platinum effect has never ceased to have its special fascination being a mixture of accident and great shrewdness. In many ways it has the attributes, say, of the detection of penicillin or that of bacteriophage. Our own interest rapidly mounted with the realisation of the alkylating-like properties of the platinum complexes, and aslo of the possibility that they might engage like classical alkylating agents in chemical cross linkage.

Listening to the accounts of clinical trial, mainly with *cis*-platinum (II) diamminedichloride it was inevitable that we should share impressions both discouraging and optimistic. I present no apologies for urging in the ultimate outcome an optimistic impression. We must remember that the progress of all such studies is inevitably slow, and that we are already aware of specific compounds vastly greater, with a chemotherapeutic index of say 200, than that of the compound which we are at the moment considering, which has an index of 8. And during these days we have learned of yet other compounds with chemical, physical and biological properties especially favourable. We therefore look forward to the future with some optimism, and I am constrained to do this from very wide experience of chemotherapeutic agents under very difficult circumstances over many years. But even so the subject in general still

remains in its infancy, and its main growth has been largely confined to the post-war years.

I am reminded that while a medical student in the University of Edinburgh—admittedly a long time ago—one was taught not merely the principles of anti-protozoal chemotherapy, but also the reasons why no anti-bacterial chemotherapy could be expected. But as you know, the entire picture was revolutionised within a very few years. Contrarywise, one tends to be encouraged at the present time by a growing understanding of spontaneous regression, and by the chemotherapeutic responses, admittedly sometimes in concert with an immune component now being observed in Burkitt's Lymphoma, in choriocarcinoma and even in acute leukaemia. I am also impressed by the growing number of cases in which the regression of various animal tumours has been attributable to chemotherapy alone.

In these closing remarks, I would be failing in my duty were I not to acknowledge our special indebtedness to great generosity on the part of Dr. Rosenberg, to similar munificence on the part of industry and to the special arrangements made on our behalf by Wadham College and Dr. R. Williams who was an ideal host.

April 1974 ALEXANDER HADDOW

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Introduction

B. Rosenberg

The medical use of metal complexes was unquestionably at its peak during the time of Paul Ehrlich during the first decade of this century. Their decline since then is due not so much to the failure of metal complexes as it is to the success of organic chemistry and biochemistry, marked by the overwhelming triumph of the sulphonamides in the 1930's followed shortly after by the discovery of the anti-bacterial antibiotics.

The bias towards organic materials is seen in the screening programme for new anti-cancer agents instituted with vigour in the mid 1950's at the National Cancer Institute in the USA. In the first 15 years of operation, of approximately 300,000 substances screened, about one half were characterised compounds. Of these only a handful were inorganic compounds and only a slightly larger number were metal organic complexes.

The discovery of the potent anti-tumour activity of platinum 4 years ago led to extensive screening of related compounds by the National Cancer Institute, the Chester Beatty Research Institute and other centres. As the results of many tests became available, a picture developed of a group of compounds with a wide spectrum of anti-tumour action and with potency as high as the best tumour chemotherapeutic agents.

This is just the beginning. With the application of quantum mechanics and ligand field theories, the chemical knowledge of metal co-ordination complexes has become quite sophisticated. At the same time there is a heightened awareness of the role of metals in normal biochemical functions. Taking all together an exciting new field is now emerging under the conglomerate title „bioinorganic chemistry".

Although there may be a reluctance to use heavy metals in man because of the widely held opinion that their soluble derivatives are general biological poisons, and although the kidney toxicity of *cis*-platinum (II) diamminedichloride has proved disappointing we know from experience that no general rules can be formulated for the biological properties of heavy metals. It is true that bare ions of heavy metals react readily and nonspecifically with nitrogen, sulphur, oxygen and other groups and can create havoc in the cell. However we are not concerned with bare ions here; we are concerned with metals locked in a tight ligand sheath, and in these, the ligand exchange reactions can be highly controlled and very specific.

A few hundred metal coordination complexes have now been tested in animals and they exhibit a rich diversity of effects and toxic levels that rival anything in the purely organic realm. For example, *cis*-platinum (II) diamminedichloride is an active anti-tumor agent in animals at concentrations ranging upwards from less than 1 mg/

kg. The LD_{50} is 13 mg/kg. The two chlorides can be replaced by a bidentate oxalato or malonato ligand and the complexes are as good or better than the first, and about an order of magnitude less toxic. Similarly, we can replace the two ammine groups by the bidentate ethylenediamine ligand and have as good or better activity than the first. Combine the two changes to make oxalatoethylenediamineplatinum (II) and suddenly we have an extremely toxic, fast acting neuromuscular poison with no anti-tumor activity even at tolerable dose levels. Now form malonatoethylenediamineplatinum (II), which has one extra carbon in the closed ring over the previous, and we again have a relatively mild, highly active anti-tumor agent. Therefore, small modifications in the molecular structure about the metal can produce radical changes in the biological effects.

Tobe, Connors and their co-workers have shown that the *cis*-dichloro-*bis*-cyclohexylamineplatinum (II) congener has an LD_{50} of over 3,200 mg/kg, while the effective curative dose (ED_{90}) for the ADJ/PC6 tumor is of the same order of magnitude as for *cis*-platinum (II) diamminedichloride. This yields a therapeutic index well over 200, one of the highest ever reported. Some of the very soluble "platinum blues" are tolerated in mice at levels in excess of 800 mg/kg. Some preliminary work in our laboratory suggests that kidney toxicity may be negligible for these latter complexes. These, and numerous other examples, force the conclusion that the generalized fears concerning the use of heavy metals, in this context, are not valid.

cis-platinum (II) diamminedichloride has dominated the scene by virtue of being chosen the first to enter into clinical trials, but it is evident that other metal coordination complexes already exist, with preferred characteristics, as potential choices for second and third generation drugs. These two new subclasses have been discovered only within the last 2 years. This suggests that many more medically interesting complexes are available for the seeking, and recommends a deeper involvement of coordination chemists in synthesizing, and submitting for testing, the widest possible variety of complexes.

One of the delights of science is to be a committed worker in, and observer of, the evaluation of a new field of research. It is doubly delightful when, as in this field, the characteristic spirit is warm cooperation and a generous sharing among the scores of scientists and the industrial companies involved. I have a deep debt of gratitude to all of these latter groups and in particular to Engelhard Industries, Matthey Bishop Incorporated, and Rustenburg Platinum Mines, Ltd. for their continued generous support of this research, and in addition to Abbott Laboratories, Engelhard Industries, Ltd. and Kyowa Hakko Kogyo Co., Ltd. for support of this Second International Symposium on Platinum Coordination Complexes in Cancer Chemotherapy. Finally, a warm note of thanks to Ms. RITTA ROSENBERG, who so beautifully organized the Symposium, and to Dr. R. J. P. WILLIAMS, our gracious host at Oxford University.

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Metal-Binding to Bio-polymers: Some Thoughts on Anti-tumour Activity

R. J. P. Williams

Introduction

The effect of a large series of platinum complexes upon growing tumour cells has now been examined. As a consequence the active anti-tumour compounds are well-separated from the inactive compounds. Unfortunately this knowledge has not yet been supported by an equivalent study either of the chemistry of platinum/biopolymer reactions or of their molecular biology. Consequently, and despite the fact that the discovery of these chemicals leads to the possibility of following anti-tumour activity with inorganic probes, we are not able to make specific statements about the functional significance of platinum at the molecular level. The worst feature of all is the decreasing effort at the micro-biological level. I return to this point later.

Firstly let me say what has been discovered by examining the binding of many metal ions to biopolymers and thence what may be especially significant about the platinum compounds.

Metal-Binding to Proteins, RNA and DNA

All metals bind to proteins at high enough concentration (dose). We shall be interested only in strong binding, i. e. binding constants $\geq 10^6$. This restriction eliminates very few metals from our discussion — only sodium, potassium and perhaps magnesium. The next step is to divide the metals of very different chemistry from one another. We shall choose to divide as follows:

a) Ca(II), UO_2(II), Ln(III), i. e. Group II A and III A of the Periodic Table and comparable cations.

b) (i) Lighter transition-metal ions such as Fe, Cu, (ii) Heavier transition-metal ions such as Pt, Ir.

c) The B-subgroup metal ions of the Periodic Table such as Zn, Pb, Hg.

Group II A and III A Metal Ions

The binding of the first division of cations is relatively easily described. The known binding sites of these metal ions taken from protein crystal structures are given in Table 1. It is invariably the case that the metals seek out multicarboxylate

Table 1. Binding sites for calcium and lanthanides

Protein	Site	Binding characteristics [a]
Carp albumin	Four carboxylate residues (Ca)	*s, f*
Thermolysin	Three carboxylate residues (Ca) There are two sites for Ca(II) and one for Ln(III)	*m, f*
Concanavilin	Two carboxylate residues (Ca)	*m, f*
Bacterial nuclease	Three carboxylate residues (Ca or Ln)	*s, f*
Insulin	Carboxylates of different chains	*w, f*
Flavodoxin	Two (?) carboxylates	*w, f*
Lysozyme	Two carboxylates	*w, f*

Notes. 1. There is a wealth of data on UO_2(II) binding showing that it too binds mainly to carboxylate groups. 2. Pb(II) falls between Group II A and Group B elements and sometimes binds to carboxylate sites. Compare the close resemblance of K and Tl(I) binding sites in subtilisin and in the chymotrypsin inhibitor.

[a] strong, *s*; medium, *m*; and weak, *w*; binding and *f*; fast exchange.

centres. There is selectivity of binding even within the division but this is based on ion size and *not* on covalence. We shall not describe these effects further at it is clear enough that platinum does not compete at these sites and that these metal ions do not compete for the prefered platinum binding sites (see below).

The A-subgroup metal ions bind to RNA and DNA at the phosphate groups. They are undoubtedly used as required cross-linking agents in tRNA and rRNA and perhaps in DNA. However their binding is weak and falls outside our discussion. Once again there is little or no reason to suppose that this binding is related to or affected by platinum binding (or vice versa) in bio-polymers, see later.

Lighter Transition-Metal Binding Sites

The sites of binding of these metal ions *in vivo* are known in a very large number of proteins, see Table 2. No general remarks about the chemical character of the sites can be made for it is clear that the metal ions can bind *very selectively*. The sites are composed of a variety of binding groups of proteins e.g. Conalbumin — phenolate oxygen and histidine nitrogen; Ferredoxin — cysteine and sulphide sulphur; Myoglobin — histidine nitrogen.

The geometries as well as the binding groups are of a large variety of types. One common feature is outstandingly important: the metal ions are bound more or less permanently and strongly only if they are bound to at least *three* protein or coenzyme groups. For first row transition metal ions such as iron, copper and cobalt it is only through the formation of several bonds that a strong enough binding centre to retain them can be built, given the low concentration of these metal ions available to biological systems. Undoubtedly platinum could compete for such centres, mercury(II) binding to them has been shown frequently, but as *exchange* is very slow indeed this possibility can be ruled out. Platinum does not act by blocking metallo-enzymes.

Table 2. Binding sites for lighter transition metals

Protein	Site	Binding characteristics [a]
Conalbumin	Nitrogen and three tyrosines (Fe)	*s, s*
Concanavilin	Histidine and carboxylate (Mn)	*m, f*
Ferredoxin	Cysteine (two) (Fe) and sulphides	*s, s*
Rubredoxin	Cysteine (four) (Fe)	*s, s*
B_{12} enzymes	5 nitrogens of coenzyme and one carbon (Co)	*s, s*
Heme proteins	4 nitrogens of coenzyme and variously nitrogen or sulphur protein ligands (Fe)	*s, s*

[a] Strong binding, *s*; medium binding, *m*; and fast exchange, *f*; slow exchange, *s*. Binding comes first.

Finally we note that these transition metals are not associated with DNA or RNA *in vivo* to any discernable degree. We will discuss the heavier transition metals later.

Lighter transition metal ions will bind *in vitro* to a large number of additional biopolymer centres. This binding is unlikely to be of much importance *in vivo* as it is usually relatively weak and to surface groups of proteins, from which the ions can exchange rapidly. The binding would be prevented by the binding of the metal ions to a large number of small molecules which effectively buffer the biological system. For example the binding of copper(II) to amino-acids will reduce very considerably the free Cu(II) concentration. We note however that should the carrier molecules and proteins for iron and copper become saturated then the effects of the free transition metal ions can be extremely deleterious. These metals act as redox catalysts as well as directly as binding agents and have a very high toxicity. Relatively speaking their binding to RNA and DNA is much less noticable than to proteins. We note that both RNA and DNA can provide two binding centres easily, but not three in contrast with the specific metal ion sites found in metallo-enzymes and metalloproteins. An outstanding result is then that proteins provide better binding groups due to their more flexible geometry than does DNA or RNA. Single stranded DNA or RNA supplies no strong binding sites.

B-Subgroup Metal Ions

The binding of zinc, Table 3, to proteins is well studied. The sites, as for light transition metal ions, are nitrogen, carboxylate and sulphur. The geometric disposition of the groups is important. Three such groups are required to hold the zinc in a protein strongly and to reduce exchange. The situation *in vitro* is very similar to that for the light transition metals. Zinc does not occur in RNA or DNA.

Mercury(II) is very different. It does not occur naturally in biopolymers of course but it binds very strongly indeed even to single sulphydryl groups. Exchange is then relatively slow. Mercurials are favourite heavy atoms for X-ray crystallography. Here we see a major distinction between heavy and light B-subgroup metal ions — extreme strength of binding to one or two sulphur (and perhaps nitrogen or even

carbon) groups can make a more or less permanent binding site for *heavy* metal ions. Exchange (on the second or minute scale) is slow. The importance of these observations becomes obvious below.

The heavy B-metals also bind to RNA and DNA. Elsewhere we have discussed this binding [5]. It involves one or two nitrogen bases, is of high binding strength, and exchange is not necessarily fast.

We can enquire "what is the difference between zinc and mercury?" The most important distinction lies in the bonding. At a very naive level of interpretation the mercury binding (and this applies to all heavy transition metal ions such as platinum)

Table 3. Binding sites for zinc

Protein	Site	Binding characteristics [a]
Carboxypeptidase	2 histidines, one carboxylate	*s, s*
Insulin	2 histidines (on each chain)	*m, f*
Carbonic anhydrase	3 histidines	*s, s*
Alcohol dehydrogenase	1 cysteine plus other groups	*s, s*

[a] Binding (first) is shown as *s* strong or *m* medium and exchange rate is shown as *s* slow or *f* fast.

is more covalent. A second feature is that the binding to mercury is stereochemically very limited. Mercury forms two linear bonds. These two factors acting together cause mercury to be a very effective blocking agent of enzyme systems for mercury is largely restricted to $-SH$ group attack. The effect of other heavy transition-metals such as Tl, Pb, As and Sb would appear to be similar in that they bind in a more covalent manner but the geometric factors are rather different. These metals too are very effective poisons (and drugs), but they bind to a much wider variety of centres.

Heavy Transition Metal Ions (Pt)

The binding of these metals, especially platinum, is now very well studied through the work of X-ray crystallographers. The compounds used $PtCl_4$, $AuCl_4$, are usually bound on the outsides of proteins. They are of course charged and they are very bulky. The main centres attacked, Table 4, are sulphur groups but nitrogen bases are also involved. The binding is through one or two protein ligands.

In proteins binding of Pt(II) to a single centre [compare mercury(II)] is sufficient to make a valuable derivative for X-ray structure determination purposes. Methionine, cysteine, cystine are possible groups. The binding is not easily reversible e. g. in ribonuclease. At higher concentrations platinum compounds bind to very many protein sites but this binding depends too on the exact nature of the platinum compound. For a variety of reasons which are discussed by others in this book, it is my opinion that although the anti-tumour agent *cis*-$[Pt(II)Cl_2(NH_3)_2]$ will attack a great variety of proteins this is not the attack which generates anti-tumour activity.

An obvious feature of platinum binding is its covalence and this it shares with mercury. However there are two marked differences between the two metal ions. Firstly there is the stereochemistry. Hg(II) forms two linear bonds while Pt(II) forms four bonds in a square. Secondly Pt(II) exchanges nitrogen groups slowly so that effectively sites on the Pt(II) can be made inert. *Cis*-$[Pt(II)Cl_2(NH_3)_2]$ is a very different reagent from *trans*-$[Pt(II)Cl_2(NH_3)_2]$. Unlike the lighter transition metals, there is *very little* flexibility in the geometry around the metal, and in order to obtain a bound slow-exchange situation to a bio-polymer *one or two* binding sites (not *three*) are sufficient. It is not surprising that strong binding of a compound such as *cis*-$[Pt(II)Cl_2(NH_3)_2]$ is very specific in biological systems for it will require a specific chelate geometry, as well as binding weakly and more generally through one group.

Table 4. $[PtCl_4]^{2-}$ Binding sites in proteins

Protein	Site
Chymotrypsin	$-S-S-$, Met, NH_2 terminal
Cytochrome-c	Met, His
Ribonuclease	Met
Subtilisin	Met, His
Carboxypeptidase	$-S-S-$, Met, His, NH_2 terminal
Human-γ-globulin	same as Hg site
Concanavilin	same as Hg site
Prealbumin	same as Hg site
Thermolysin	His
Triose phosphate isomerase	same as Hg site $-SH$ and other sites

Notes. 1. High concentration of amines can be used to remove Pt from a binding site in some proteins. 2. The site attacked depends upon the buffer medium in which the Pt is dissolved. Pt compounds bind to many simple groups such as phosphates. I am grateful to Prof. D. C. PHILLIPS and Dr. G. PETSKO for help in compiling this table. 3. There is some data to show that elements such as Pd, Ir, Au(III) bind in a similar way to Pt.

Table 5. The nature of coordination compounds in biology

Metal ion	Type of ligand as partner	Number of partners for strong binding	Exchange rate from site	Stereochem. demand
Na, K	Oxygen (O)	$\geqq 4$	Fast	Nil
Mg, Ca	Oxygen anions	$\geqq 4$	Medium	Nil
Zn	O, N, S	$\geqq 3$	Slow	Little
Fe, Co	O, N, S	$\geqq 3$	Slow	Medium
Hg	N, S	2.1	Slow	Strong
Pt	N, S	2.1	Slow	Strong
Carbonium ion	N, S, (O)	1	Slow	Strong

Note. The above table applies to exchange from a protein. It would be unwise to use it as more than a general guide but it does show how the chemical ideas currently used in the understanding of organic mechanisms (last line) are closely parallel to those needed for an understanding of platinum chemistry.

Through the work of a large number of research groups, reported later in the symposium, it seems likely that *cis*-[Pt(II)] is a cross-linking reagent for DNA. It is clear that if such cross-links are to be effective the Pt(II) compound formed must have kinetic stability. Reaction with basic nitrogen atoms in DNA would give such stability but this reaction also produces a product which has a particular structure. Such an attack would be difficult to mimic with other metal complexes which are not closely related to Pt(II) despite the fact that many metals will attack the basic nitrogen atoms in one way or another. We stress the reasons again, Table 5.

a) Pt(II) will bind strongly when bound through *two* nitrogen residues.

b) The binding is likely to be only *slowly* reversible.

c) Stereochemical selectivity is built in to the reagent as the two NH_3 groups of $[(NH_3)_2PtCl_2]$ will not exchange.

d) Pt(II) has common reactions with other compounds which attack DNA, Table 5.

Given that the site of attack is specific, how does the platinum find the site? What could this specific (critical) site be?

Entry of Pt(II) into Cells

Our knowledge of the activity of the Pt(II) compounds is also hindered by a lack of knowledge of a great deal of Pt(II) chemistry with biologically interesting groups. Before any attack on DNA can occur the *cis*-dichloro-compound of Pt(II) is likely to undergo multiple rapid substitutions. It could well be that in the high chloride medium of the blood stream the reactions are only the exchange of one chloride for another. In cells the chloride ion concentration is much lower and under such conditions chloride will be replaced by such groups as phosphate anions. Again, passage across the cell membrane may be very restricted for an ion such as $[Pt(H_2O)_2(NH_3)_2]^{2+}$, (while $[PtCl_2(NH_3)_2]$ and such molecules as $[PtCl_2(NH_2R)_2]$ may penetrate readily), but inside the cell it could well be that the dichloride is not the strongest electrophile. We can make few comments about these points as yet. I believe that Pt-phosphate bonding inside cells also needs to be considered. Its exchange would be moderately fast.

Why Platinum?

The question as to why it is platinum that is active, and platinum in a particular form, is now answerable using the summary table, Table 5. A set of chemical parameters is optimised somewhere close to platinum in the periodic table but complexes of elements such as Au, Hg, Ir, Rh, Os, Ru, Re in particular could form compounds which might mimic *cis*-$[Pt(NH_3)_2Cl_2]$ quite well, although it could well be difficult to find a very close relative of this particular metal complex using another metal.

Now while we may suppose that it is the cross-linking function of the bi-functional platinum compouds which leads to anti-tumour activity (see later discussion in this symposium) it would be a mistake to classify the platinum drugs together with other bi-functional reagents such as the di-alkylating agents also used to cross-link DNA. Undoubtedly there are many similarities in the general pattern of activity of

the two series of drugs but the differences in chemistry and stereochemistry are very large indeed. In fact I do not regard cross-linking as a readily definable term. In order to bring out this point and to establish an attitude of mind to "cross-linking" I must first give a very brief comparative account of the way in which I see platinum drugs in relationship to other drugs used in cancer chemotherapy.

A Comparative Approach to Platinum Activity

In this final section I shall try to stimulate some discussion as to why the action of these platinum compounds is so biochemically specific. I want to stress the importance of microbiological studies. I shall start from the general premise that induction of provirus (lysogeny) and anti-tumour activity have much in common, but

Table 6. Groups which attack DNA

Alkylating agents	Epoxides, alkyl halides (note also Pt), Nitrosoalkylureas, sulphur and nitrogen mustard.
Intercalating agents	Polycyclic hydrocarbons, sterols, Mitomycin c, nalidixic acid.
Radical agents	$OH^{\cdot}$ etc., from NH_2OH, H_2O_2, etc., *hv*, X-rays.
H-bond agents	Peptides such as Distamycin.
Biological incorporation	Bromouracil etc.

that agents which are effective to some extent in both activities have very variable effects in (i) stimulating elongated (filamentous) growth of bacterial cells, (ii) causing mutation. In the Tables 6, 7 and 8, I have attempted to make some comparative remarks about different chemicals which have these effects. From the Tables it is clear that filamentous growth is only to be associated with anti-tumour activity in a very vague way and there must be different ways of causing filamentous growth both related to and dissociated from any effect on DNA. (It was merely fortunate that ROSENBERG discovered that the platinum compounds act as anti-tumour agents because he observed filamentous growth in *E. Coli.*) Again mutagenic agents must act at the level of DNA, yet clearly effective mutagenic agents are not good anti-tumour agents nor do they cause pro-virus induction very effectively. LOVELESS [3] pointed to the fact that the most powerful inducing agents were usually the poorest mutagens and *vice versa,* although nearly all agents which had one effect also showed the other to some degree, Table 9.

As I see it, the platinum compounds fall in the group of good inducers and poor mutagens, although the last part of the statement is based upon the absence of evidence for mutagenic effects. This division of the compounds corresponds closely with the distinction between two types of agent which attack DNA — those that "cross-link" and those that do not, Table 10. I conclude from the nature of the chemicals that good "cross-linking" agents are poor mutagens but good inducers, whereas good mutagens attack single bases and do not act as inducers. I believe then that platinum is again in the first group. To improve the general line of this discussion I have

Table 7. Properties of compounds which interact with DNA *

Agent	Anti-tumour	Muta-genic	Carcino-genic	Prophage produced	Fila-ments	Synthesis of colicin *
UV	?	++	+	+	+	+
X-rays	+	+	+	+		−
Di-(2-chloroethyl)-methylamine	+	+	+	+	+	+
2-chloroethyl-dimethylamine	(0)	0	0	0	−	−
H_2O_2 in inorg. media		0	−	0		−
H_2O_2 in org. media		(+)	−	+	?	+
t-Bu-O_2H		(+)	+	+		+
2,4,6-*tris*-ethyleneimino-1,3,5-triazine		+	+	+		−
Butadiene-1:3-diepoxide		+	+	+		−
Ethylurethan Water soluble		+	+	0		−
1:4-dimethanesulphonyloxy-butane		−	+	0		−
9:10-dimethylbenzanthracene	0	+	+	0		−
2-acetaminofluorene	0	+	+	0		− Not
Methylcholanthrene	0	+	+	0		− water
β-naphthylamine	0	+	+	0		− soluble
benzidine	0	+	+	0		−
$Pt(NH_3)_2Cl_2$	+	? −	? −	+	+	?

* From LWOFF [4].
* Highly specific proteins which kill other bacilli.

drawn up in Table 10 lists of reagents indicating the *strength of their cross-linking* ability. It is a simple matter to compare this list with mutagenic action or with lysogeny. Could it be that the effective anti-tumour agents are just those which arrest DNA synthesis most effectively? This could be brought about either through direct attack on DNA (Pt), or through attack on the cell membrane for a cell is an intercommunicating system. For my part I feel that the biochemistry of the above compounds is too selective to represent an attack on chemical groups in the membrane.

When comparing the agents which produce cancer with those that "cure" it I have often heard quoted a statement called Haddow's Paradox — "The agents which cause cancer also cure it" [2]. Whether Haddow ever said precisely this or not I have been unable to ascertain but it should not be generally true. It seems to me that cancer producing and anti-cancer agents should differ in much the way that mutagens differ from phage-inducing agents. The difference will rest in the *strength of their cross-linking*, i. e. there should be a difference of degree if not of kind.

There should then be a wide spectrum of reagents which will be effective in both tacks, but the extremes of the spectrum will be represented by anti-tumour agents