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INORGANIC CHEMISTRY AND DRUG DESIGN

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A huge world of inorganic drugs—which has been ill-explored since organic chemists dominate biochemical practice—is slowly opening (1).

I. Introduction

Most of the elements of the Periodic Table up to and including bismuth ($Z = 83$) are potentially useful in the design of new drugs and diagnostic agents. The radioactivity associated with elements of higher atomic numbers poses serious toxicity problems, although a few

radioisotopes of lighter elements with short half-lives are effective at low doses for diagnosis and therapy.

Both essential and nonessential elements can be used in drugs (*2–4*). At least 25 elements are now thought to be essential for animal life (Table I), a requirement that has presumably arisen by natural selection based on availability and advantages to animals (*5*). The current list may not be complete; the recent work of Nielsen (*6*) has strongly suggested that boron should be added to the list, because it appears to prevent some bone diseases. Unfortunately, our understanding of the natural biochemistry of the essential elements is too poor to enable the rational design of compounds to control the metabolism of many of them; for example, we know little about how Cr, V, Mn, Ni, and Mo are absorbed, transported, and stored in the body. Is silicon required to strengthen connective tissues such as the aorta via polysaccharide crosslinks or merely to protect against the harmful effects of aluminum (*7*), a nonessential element?

The metabolism of the essential elements can be controlled by pharmaceutical agents in two ways: either by the supply of specific compounds with targeting properties that ensure delivery or removal of the element from particular organs, or by interfering with the natural biochemical pathways for that element. This can often be done with organic drugs, for example, certain steroids induce the synthesis of Ca-binding proteins. More and more it is becoming apparent that intimate feedback mechanisms exist in nature among inorganic elements and

TABLE I

ELEMENTS ESSENTIAL FOR WARM-BLOODED MAMMALS[a]

H																	He
Li	Be											**B**	**C**	**N**	**O**	**F**	Ne
Na	**Mg**											Al	**Si**	**P**	**S**	**Cl**	Ar
K	**Ca**	Sc	Ti	**V**	**Cr**	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	Ga	Ge	**As**	**Se**	Br	Kr
Rb	Sr	Y	Zr	Nb	**Mo**	Tc	Ru	Rh	Pd	Ag	**Cd**	In	**Sn**	Sb	Te	**I**	Xe
Cs	Ba	La*	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	**Pb**	Bi	Po	At	Rn
Fr	Ra	Ac**															
		*	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	
		**	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr	

[a] Twenty-five elements (underlined) are thought to be essential and it seems likely that boron will soon be added to these (*6*). A further three (Cd, As, and Pb) are potentially essential, in that animals lose body weight when the traces of these in the diet are removed (*2*). It is possible that silicon is essential only to prevent the harmful effects of aluminum (*7*).

organic compounds. So too must organic and inorganic pharmacology progress hand in hand in the future.

The wider use of inorganic compounds in health care depends heavily on overcoming "the toxicity problem." Arsenic, for example, is widely perceived to be a toxic element, but the enormous variation in toxicity among the types of arsenic compounds is not widely acknowledged: As(III) compounds are usually much more toxic than As(V) compounds, and arsenobetaine ($Me_3As^+CH_2CO_2^-$) is relatively nontoxic, which is fortunate because man consumes it regularly in certain fish and crustacea (e.g., lobsters). This illustrates one of the main points that we have to establish beyond doubt: that inorganic compounds can be *designed* to have a specificity of biological activity. Natural biochemical processes even use extremely hazardous inorganic compounds (e.g., cyanide, oxides of nitrogen, sulfide, and hypochlorite) in a carefully controlled way. So too can pharmacology.

It is worth recalling the Bertrand diagram (given in Ref. *8*, but a term coined by B. L. Vallee): for essential elements, very low doses may give rise to deficiency diseases; a certain daily minimum dose is required for the optimum physiological response, and at higher doses toxicity will arise. For Zn, about 10–15 mg is required daily, but high doses of zinc sulfate can be lethal. However, to define the zinc content of the dose is not enough; zinc complexed to phytic acid (inositol hexaphosphate, a common constituent of plant seeds) in the diet is not absorbed. The placement of a curve for an element on the Bertrand diagram will therefore depend not only on the element but also on the particular compound, its route of administration, and direct or indirect interactions with both organic and other inorganic compounds. Copper deficiency, for example, can cause anemia, an apparent defect in Fe metabolism. This is a complicated situation that presents many challenges for inorganic chemistry.

For nonessential elements, low doses may often be tolerated without deleterious effects (resistance), but higher doses are toxic. It is in the low-dose region that nonessential elements can be pharmacologically useful, perhaps for killing an invading microorganism. It is notable that many "nontoxic" inorganic compounds are used on the thousands-of-kilograms scale each year by pharmaceutical industries as vehicles for drug delivery (Table II). Little attention is paid to their chemistry in the products. Modern solid-state inorganic chemistry might be able to play a role in improving the surface adsorption or activation properties of some of these agents.

The use of inorganic compounds in medicine dates back to ancient times. The Egyptians are said to have used copper to sterilize water in

TABLE II

SOME INORGANIC MATERIALS USED AS EXCIPIENTS FOR TABLETS, CAPSULES, OR SUSPENSIONS

Calcium phosphate	Titanium dioxide (white opacifier)
Sodium chloride	Calcium carbonate (solid base)
Sodium metabisulfite (antioxidant)	Sodium phosphate (buffer agent)
Silicon dioxide	Talc (magnesium silicate)
Magnesium aluminium silicate (suspending agent)	Iron oxide (coloring agent)

3000 BC and the Chinese were using gold in medicine in 2500 BC; mercurous chloride was known to be a diuretic during the Renaissance period, and mercurial diuretics were widely used up until the 1950s. It was not until the beginning of this century that respectable inorganic chemistry was introduced into pharmacology: Ehrlich's 606th arsenic compound, arsphenamine, provided a successful treatment for syphilis, and gold cyanide was effective for tuberculosis following the microbiological work of Koch. In 1912, Vianna introduced antimony compounds for treating the parasitic disease leishmaniasis, and in 1929 gold compounds were first used by French physicians to treat rheumatoid arthritis, a practice that is still widespread today.

However, even today, the chemistry of both gold and antimony drugs is not well understood. It is important that modern drugs be adequately characterized and future progress depends on advances in understanding the mechanisms of action of inorganic compounds; more emphasis must be placed on identifying target sites. In turn, new methods are required for investigating ligand exchange and redox reactions of inorganic compounds in biological media. Arsenic, antimony, bismuth, and gold, for example, are particularly difficult elements to study in solution: none of these has useful NMR isotopes. The metal species that reaches a biological target site is likely to be different from that which is administered, and both kinetic and thermodynamic aspects of the reactivity are equally important. Indeed, biological systems are rarely at thermodynamic equilibrium: potential metal ligands are constantly being synthesized and transported in and out of compartments. Organic ligands may also be activated by metabolism and covalent modification *in vivo*. Biological media have a wide variety of dielectric constants; some are extremely hydrophobic (lipid membranes) and some are hydrophilic (extracellular fluids), and this influences the choice of model systems for the study of inorganic drugs. Testing of inorganic compounds too has to be undertaken with some

TABLE III

SOME INORGANIC PHARMACEUTICAL AND DIAGNOSTIC AGENTS WITH EXAMPLES OF TRADE OR OTHER COMMON NAMES

Element	Product	Compound	Use
Li	Camcolit	Li_2CO_3	Manic depression
B	—	$B_{12}H_{11}SH^{2-}$	Neutron capture therapy
	—	Sodium perborate	Antiseptic (gargle)
N	Laughing gas	N_2O (nitrous oxide)	Anesthetic
	—	$NaNO_2$	Vasodilator (relaxant)
F	—	SnF_2	Tooth protectant (pastes)
Mg	Magnesiocard	$Mg(II)(L\text{-}Asp)_2 \cdot 2HCl \cdot 3H_2O$	Nutritional supplement
	Magnesia	$Mg(II)O$	Antacid, laxative
	Epsom salts	$Mg(II)SO_4 \cdot 7H_2O$	Laxative (Epsom salts)
Al	—	$Al(III)(OH)_3$	Antiperspirant, antacid
	Alum	$KAl(SO_4)_2 \cdot 12H_2O$	Astringent
Si	Kaolin	$Al_2(OH)_4Si_2O_5$	Antidiarrheal
	Talcum powder	$Mg_3(OH)_2Si_4O_{10}$	Mild antiseptic
S	Thiosulfate	$Na_2S_2O_3$	Cyanide antidote
Cl	Eau de Javelle	KClO (hypochlorite)	Disinfectant
Ca	—	$Ca(II)CO_3$	Antacid
Fe	—	Fe(II) fumarate, succinate	Supplement
	Nipride	$Na_2[Fe(II)(NO)(CN)_5]$	Hypotensive (vasodilator)
Co	Cobaltamin S	Coenzyme vitamin B_{12}	Supplement
Cu	—	$Cu(II)CO_3$	Supplement
Zn	Calamine	$Zn(II)O$	Skin ointment, astringent
	Omadine	Zn(II) pyrithione	Antimicrobial (antidandruff)
	—	Zn(II) undecanoate	Antifungal (athlete's foot)
	Calamine lotion	$Zn(II)CO_3/Fe(III)_2O_3$	Skin ointment
		Zn(II) citrate	Antiplaque (toothpaste)
Ge	Germanium-132	Carboxyethyl Ge(IV) sesquioxide	Anticancer, immune adjuvant, hypotensive
As	—	$As(III)_2O_3$ (arsenous acid)	Skin, blood disorders (veterinary)
Se	—	Selenium sulfide	Antiseborrheic
Br	—	NaBr	Sedative
Sr	—	$Sr(II)(acetate)_2$	Toothpastes
Zr	—	Zr(IV) glycinate, lactate	Antiperspirant
Tc	Ceretec	$^{99m}Tc(V)$ propyleneamineoxime	Diagnostic radio imaging
Ag	Flamazine	Ag(I) sulfadiazine	Antibacterial
Sn	Sn-heme	$Sn(IV)(protoporphyrin)Cl_2$	Treatment of jaundice
Sb	Triostam	Sodium Sb(V) gluconate	Antileishmanial
I	—	I_2	Antiinfective, disinfectant
Xe	—	Xenon gas	Experimental anesthetic
Ba	Baridol	$BaSO_4$	X-Ray contrast medium
Ce	—	$Ce(III)(NO_3)_3$	Antibacterial (burn wounds)
Gd	Magnevist	$[Gd(III)DTPA](meglumine)_2$	MRI contrast
Pt	Cisplatin	*cis*-$Pt(II)Cl_2(NH_3)_2$	Anticancer
	Carboplatin	$Pt(II)(CBDCA)(NH_3)_2$	Anticancer
Au	Myocrisin	Disodium Au(I)(thiomalate)	Antiarthritic
	Solganal	Au(I)(thioglucose)	Antiarthritic
	Auranofin	$Au(I)(PEt_3)$(acetylthioglucose)	Antiarthritic
Hg	Mersalyl, Mercurin	Alkyl Hg(II)(OH)	Diuretic
	—	Phenyl Hg(II) nitrate	Antimicrobial
Bi	De-Nol	$K_3[Bi(III)(citrate)_2]$	Antacid, antiulcer

care: compounds may undergo transformations in cell culture media before they enter cells.

Discussed in this article are some of the current uses of inorganic compounds as drugs and diagnostic agents, attempting to highlight some of the above points. I focus especially on areas of demonstrated medical and clinical interest (Table III) and I include organic agents if their activity is in some way dependent on inorganic chemistry. The examples chosen are illustrative and do not form a comprehensive list, and are mostly concerned with metals. For convenience I have used the classification shown in Table IV. In the first section are agents for which it appears to be essential that at least some part of the administered compound remains intact at the target site, whereas in the second section the nature of the original ligands is less important—the metal ions are usually kinetically labile—although the ligands may have a major influence on absorption and distribution of the drug. In the third section, one of the functions of the metal may be to deliver an active ligand to a target site, and here I include organic drugs that

TABLE IV

CLASSIFICATION OF INORGANIC DRUGS AND DIAGNOSTIC AGENTS

1. Active complexes	
Cr, Co, Rh (neuromuscular blocking agents)	Al, Zr (antiperspirants)
Pt, Ru (anticancer agents)	Ba (X-ray contrast)
Gd (NMR contrast agents)	Sn (jaundice)
Co (vitamin B_{12})	
2. Active elements	
Li (manic depression)	Au (rheumatoid arthritis)
F, Sr, Sn (toothpastes)	Sb (leishmaniasis)
Ag, Hg (antimicrobial agents)	^{10}B (neutron capture therapy)
^{99m}Tc, ^{111}In (radiopharmaceuticals)	
3. Active ligands	
a. Delivered by a metal	
Ca, Mg, Al (antacids)	Bi (antiulcer)
Fe (antihypertensive)	Ti, Au (anticancer)
b. Delivered to a metal	
Bleomycin (Fe)	Desferrioxamine (Fe, Al)
Penicillamine (Cu)	Bisphosphonates (Ca)
c. Metalloenzyme inhibitors	
Angiotensin-converting enzyme (Captopril, Zn)	
Ribonucleotide reductase (hydroxyureas, Fe)	
Lipoxygenase (acetohydroxamates, Fe)	

require a metal for activity, and agents that are targeted on metals in enzymes. This is not intended to be a rigid classification; some agents can be placed in more than one class and others are placed out of ignorance of their mechanisms of action.

II. Active Complexes

A. Neuromuscular Blocking Agents

Low-spin d^6 transition metal complexes are classical examples of kinetically inert complexes. When injected into mice, species such as $[Co(NH_3)_6]^{3+}$, $[Fe(1,10\text{-phen})_3]^{2+}$, $[Ru(bipy)_3]^{2+}$, and $[Os(terpy)_3]^{2+}$ rapidly cause convulsions, paralysis, and death by respiratory failure. They produce a curariform block at the neuromuscular junction, consistent with inhibition of acetylcholine esterase. The *d* isomers of $[Ru(phen)]^{2+}$ and $[Os(phen)]^{2+}$ are 1.5–2 times more potent than the *l* isomers (*9,10*). These inert complexes are excreted largely unchanged from the body.

The fluorescent complex $[Ru(II)(3,4,7,8\text{-tetramethylphen})_3]Cl_2$ is readily taken up by P388 leukemia cells in culture and is visible on the cell surface, in the cytoplasm, and in the nucleus, but it does not exhibit antitumor activity *in vivo* (*11*). The platinum complexes [Pt(en)(oxalate)], $[Pt(NH_3)_2(H_2O)_2]^{2+}$ (as the dinitrato complex), and $[Pt(en)_3]^{4+}$ also cause convulsions in animals (*12*); the latter two complexes are positively charged and relatively inert and the former is neutral and presumably undergoes an activation step before it binds to the neuromuscular junction. Curiously, the related malonato complex is not a neurotoxin. Care is taken in the clinic to administer cisplatin in saline solutions to avoid hydrolysis and minimize the production of neurotoxic aqua or hydroxobridged Pt(II) species.

B. Platinum Anticancer Drugs

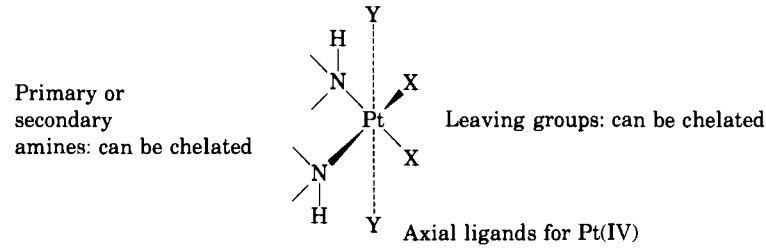
There is a wide spectrum of biological activity among platinum complexes, including the neurotoxins discussed above; the reactive protein cross-linking agents $[Pt(II)Cl_4]^{2-}$ and $[Pt(IV)Cl_6]^{2-}$ (probably activated *in vivo* by reduction), which can induce potent immunological reactions at doses as low as 10^{-15} g; relatively nontoxic complexes such as $[Pt(NH_3)_4]^{2+}$; and *cis*-$[PtCl_2(NH_3)_2]$, the anticancer agent, the trans isomer of which is inactive.

With just a few exceptions, active platinum antitumor agents contain Pt(II) in a square–planar configuration with two cis primary or secondary amines, together with two other more weakly bound ligands such as chlorides or carboxylates (*13–17*) (Table V). Related Pt(IV) complexes with additional chloro or hydroxo axial ligands are often also active and are believed to be reduced to Pt(II) *in vivo* with removal of the axial ligands.

Cisplatin (**1**) is effective against testicular tumors, ovarian carcinoma, and some other types of cancer, but exhibits poor activity against breast and lung cancers. It is a very toxic complex, and common side effects include loss of high-frequency hearing, neuropathy, and nausea. Kidney damage is minimized by hydration of patients to ensure fast passage of free drug through the kidneys. Cisplatin readily hydrolyzes in water with a half-life of ~1.7 hr at 37°C. The aqua

TABLE V

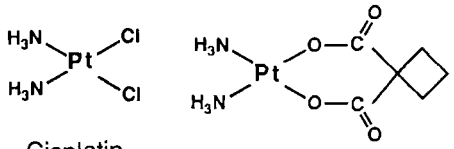
PLATINUM ANTICANCER DRUGS: STRUCTURE–ACTIVITY RELATIONSHIPS AND COMPLEXES ON CLINICAL TRIALS



Complex[a]	Status
cis-$PtCl_2(NH_3)_2$	Registered drug: cisplatin
Pt(1,1-CBDCA)$(NH_3)_2$	Registered drug: carboplatin (paraplatin)
Pt(IV)$Cl_2(OH_2)(i\text{-}C_3H_7NH_2)_2$	Phase 2 trials (iproplatin)
Pt(isocitrate)(1,2-dach)	Trials
Pt(TMA)(1,2-dach)	Trials
Pt(oxalate)(1*R*,2*R*-dach)	Trials
Pt$(SO_4)(H_2O)$(1,1-damch)	Trials
Pt(malonate)(en)	Trials
Pt(pyruvate)$_2$(1,2-dach)	Trials

[a] Abbreviations: 1,1-CBDCA, 1,1-cyclobutanedicarboxylate; dach, 1,2-diaminocyclohexane; TMA, 1,2,4-benzenetricarboxylate; 1,1-damch, 1,1-diaminomethylcyclohexane; en, 1,2-diaminoethane.

complexes (pK_a values of ~5.6 and 7.3) are considerably more reactive than chloro or hydroxo complexes and it is generally believed that this provides an activation mechanism for cisplatin inside the cells, where the chloride concentration (~4 m*M*) is much lower than it is outside the cells (~103 m*M*). Carboplatin (**2**) (paraplatin) does not undergo



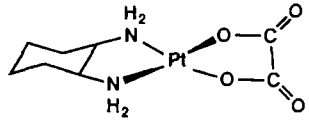
Cisplatin

1

Carboplatin

2

significant hydrolysis in water and its lower toxicity means that higher doses have to be administered to achieve cure rates similar to those of cisplatin; also, paraplatin does not cause loss of high-frequency hearing. Several other complexes are undergoing clinical trials (Table V), including 1,2-diaminocyclohexane complexes, such as **3**, which are usually not cross-resistant with cisplatin.



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How does platinum kill a cell? A major target appears to be the N7 of guanine exposed in the major groove of right-handed, double-helical B DNA. The formation of an intrastrand G–G crosslink, as in the model complex *cis*-[$Pt(NH_3)_2${d(pGpG)}], disrupts base–base stacking interactions by forcing open the dihedral angle between the guanine base planes to ~180°. It also induces a change in the pucker of the 5′ sugar ring from C2′ to C3′ endo, and introduces NH–phosphate H bonding (*18*). This produces a kink in the DNA helix (*15*), which is probably not recognized by the repair enzymes in tumor cells, whereas it is in normal cells. The isomer *trans*-[$PtCl_2(NH_3)_2$] cannot form a DNA cross-

link with the same geometry, which could account for its inactivity. However, as well as these thermodynamic aspects it is also necessary to consider the role of kinetics and ammine displacement in both the mechanism of antitumor activity of cisplatin and its side effects. Because biological systems are rarely at thermodynamic equilibrium, the fastest reactions may be those of most consequence. Part of the biological discrimination among the isomers could be analogous to the chemical Kurnakov test (*19*), which uses thiourea to convert the cis isomer to the yellow product $[Pt(thiourea)_4]^{2+}$ (ammine ligands displaced], whereas the trans isomer forms the white product *trans*-$[Pt(thiourea)_2(NH_3)_2]^{2+}$ (ammine ligands retained), reactions that are driven by the high kinetic trans influence of S-bound ligands, weakening the trans bond.

It was evident from our early work (*20*) on reactions of cisplatin in dimethylsulfoxide [DMSO—a solvent that has frequently been used during testing *in vitro* (*21*)] and with the enzyme ribonuclease (*22*) (which has a surface methionine, Met 29), that sulfur ligands can readily induce the release of ammonia ligands, and even chelated diamines. Moreover, under typical incubation conditions for tests in cell cultures (Dulbecco's minimal medium, 24 hr, 37°C), it can be shown (*23*) by ^1H NMR that methionine in the incubation medium readily reacts with cisplatin or its ethylenediamine (en) analogue (Fig. 1). Using similar methods, such reactions can also be demonstrated in blood plasma (*24*), and ammonia release is a major event when the rescue agent (removal of excess Pt from the body) diethyldithiocarbamate is added to cisplatin in plasma *in vitro*. Cisplatin can also bind to critical methionines of the plasma protein α_2-macroglobulin and α_1-proteinase inhibitor (antitrypsin) (*25*), and $[Pt(L\text{-}Met)_2]$ has been isolated from the urine of patients treated with cisplatin (*26*). Using radiolabeled Pt and ligand, Robins and co-workers have noted that dissociation of Pt from ethylenediamine can be observed as soon as 1 hr after administration of $[Pt(en)Cl_2]$ to animals (*27*), and that the released en can be further metabolized and its carbon incorporated into DNA. Our recent work (J. D. Ranford and P. J. Sadler, unpublished) suggests that methionine can activate cisplatin in reactions with 5′-GMP.

Release of ammonia from cisplatin inside red cells *in vitro* on reaction with intracellular glutathione (GSH, ~2 m*M*) can be detected by NMR studies on intact cells (*28*); initially, displacement of Cl^- occurs, giving *cis*-$[Pt(SG)(Cl)(NH_3)_2]$, and finally a 1:2 Pt:GSH polymer. The trans isomer reacts more rapidly, but forms the stable product *trans*-$[Pt(SG)_2(NH_3)_2]$.

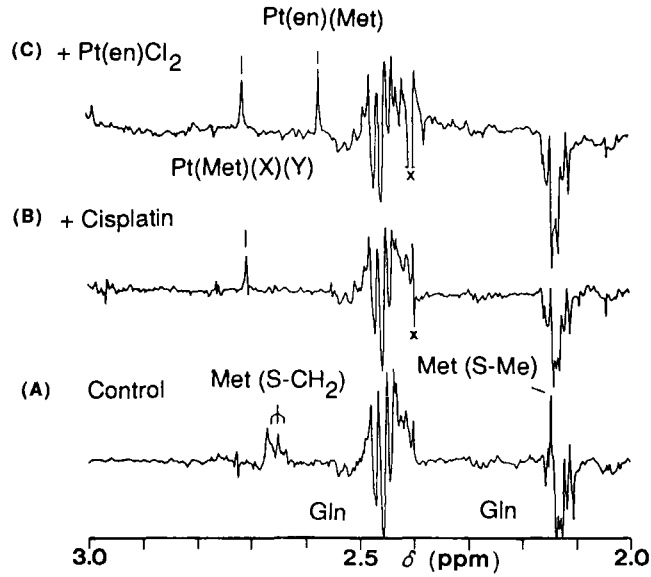


FIG. 1. The 500-MHz Hahn spin-echo ^1H NMR spectra of a cell culture medium, Dulbecco's minimal essential medium, before (A) and after (B) reaction with 400 μM cisplatin for 24 hr at 37°C, and after (C) reaction with $[Pt(en)Cl_2]$. Note the disappearance of the singlet at 2.14 ppm for the *S*-methyl of L-Met, which has formed Pt–Met complexes, giving new peaks at ~2.7 ppm. (Adapted from Ref. *23*.)

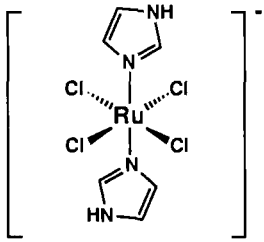
Tumor cells with acquired resistance to cisplatin (a major clinical problem) are known to over-express metallothionein, a protein of ~61 amino acids, of which 20 are cysteines. Metallothionein is involved with the natural metabolism of Zn(II) and Cu(I), and all the Cys sulfurs are involved in forming terminal or bridging bonds to the 7–10 metals that form clusters on the protein. Petering *et al.* (*29*) have recently shown that en is rapidly released from $[Pt(en)Cl_2]$ on reaction with metallothionein *in vitro* and that the rate-determining step does not involve aqua or hydroxo intermediates.

Further consideration must therefore be given to the role of amines as leaving groups. It would be of interest to determine the order of leaving of the N bases from positively charged complexes of the type *cis*-$[PtCl(N\text{-donor})(NH_3)_2]^+$, where the N donor is, e.g., pyridine. These complexes are active against S180a, P388, and L1210 tumors in mice (*30*). The leaving ability of pyridine may be assisted by its low pK_a (5.25), and it is notable that the second pK_a of diamines such as en (pK_a values of 10.71 and 7.56) is low.

C. Ruthenium Complexes

Other transition metal complexes with cis dichloro ligands have been tested for antitumor activity. The palladium analog *cis*-$[PdCl_2(NH_3)_2]$ is inactive, probably due to the high kinetic lability of Pd(II) compared to Pt(II), and isomerization is facile; this process is blocked in the chelated en complex, which is active (*16*). More inert metal ions such as Rh(III) and Ru(III) also have active analogs (Table VI), and it is likely that some ruthenium complexes will soon enter clinical trials (*31–33*).

The Ru(III) complex *fac*-$[RuCl_3(NH_3)_3]$ is highly active against P388 leukemia but is not very soluble. The related complexes *trans*-[Ru(III)Cl_4I_2](IH) (**4**), where I is imidazole or indazole, have improved solu-



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bility and may soon enter trials for the treatment of colon cancer, for which cisplatin is not effective. Both Ru(III) and Ru(II) states may play a role in the activity of these complexes, because the redox potentials of many ruthenium complexes are poised around 0 eV and *in vivo* reduc-

TABLE VI

Activity of Some Ruthenium Complexes against P388 Leukemia[a]

Complex	Comment on activity
fac-[Ru(III)$(NH_3)_3Cl_3$]	High (but insoluble complex)
trans-[Ru(III)$(Im)_2Cl_4$]ImH	High (high-activity colorectal cancer)
cis-[Ru(III)$(NH_3)_4Cl_2$]Cl	Moderate
[Ru(III)$(Im)Cl_5$]$(ImH)_2$	Moderate
cis-[Ru(II)$(DMSO)_3(DMSO)Cl_2$]	Marginal (high-activity Lewis lung carcinoma)
[Ru(II)$(bipy)_2(ox)$]	Low (kinetically inert complex)
$[Ru_3O_2(NH_3)_{14}]^{6+}$ (ruthenium red)	Low

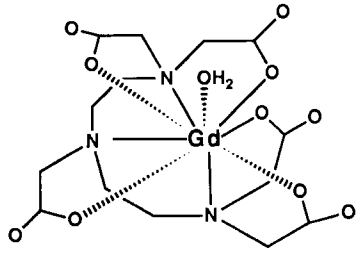
[a] Based on data from Refs. *31–33*.

tion may be a requirement for activity on account of the extreme kinetic inertness of Ru(III). Ruthenium red, $[Ru_3O_2(NH_3)_{14}]^{6+}$, is a well-known inhibitor of calcium uptake by cells and organelles and is a cytologically useful stain for negatively charged polysaccharides, but it is not a very active antitumor agent, presumably because it does not penetrate cell membranes well.

Large numbers of other metal complexes also exhibit anticancer activity, and this field is beginning to be explored in depth (*34*).

D. Contrast Agents for X-Ray and NMR Imaging

Many barium salts are toxic, but the extreme insolubility of $BaSO_4$ in water (~10 μM) allows its safe use as a contrast agent for X-ray imaging of the gastrointestinal tract. Contrast in NMR images of the body can be achieved by selective perturbation of the relaxation times of protons (usually water) with paramagnetic or ferromagnetic materials (*35*). The most effective paramagnetic metal ion is Gd(III), with its seven unpaired $4f$ electrons and long electron spin relaxation time. Simple salts of Gd(III) are too toxic for human use, but chelated complexes such as $[Gd(III)DTPA]^{2-}$ (**5**) (where H_5DTPA is diethylene-



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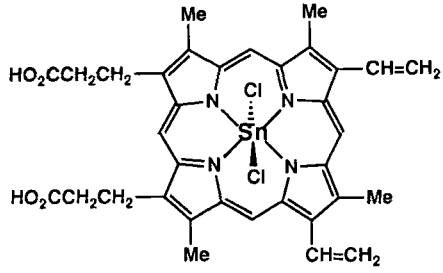
triaminepentacetic acid), which still has one vacant coordination site for H_2O, are rapidly excreted through the kidneys into the urine and can be injected safely in gram quantities. Sich large amounts are required in order to achieve local levels of > ca. 50 μM necessary for observable NMR contrast. Soluble polysaccharides such as dextran, or insoluble ones such as starch and cellulose, can be labeled with polyaminocarboxylates and are also effective contrast agents (*36*). Magnetite particles tend to undergo phagocytosis and be concentrated in organs such as the liver (*37*). Manganese(II) salts (high-spin $3d^5$) can also provide effective NMR contrast, but many types of these complexes appear to end up in the liver, perhaps entering natural meta-

bolic chains for this essential metal, which is readily oxidized to Mn(III).

E. Cofactors and Prosthetic Groups

Dichloro(protoporphyrin IX)tin(IV) (Sn–heme, **6**) is undergoing clinical trials for the treatment of neonatal jaundice. It is a potent inhibi-

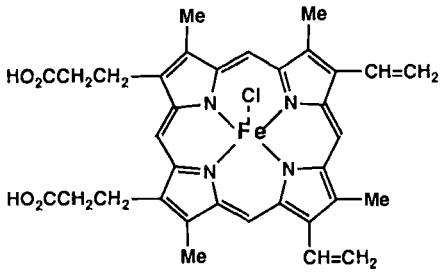


Sn-haem

6

tor of the enzyme heme oxygenase, which is involved in the rate-determining step of the degradation of iron protoporphyrin IX to bilirubin. If bilirubin is not detoxified quickly enough by the liver, it gives rise to jaundice. Sn–heme is a potent inhibitor of heme oxidase activity in the liver, spleen, kidney, and skin, and can rapidly reduce the amount of bilirubin in the bloodstream (*38*).

Hemin itself (**7**) is a positive modulator of the genes for heme oxygenase, as well as cytochrome P-450, globin, and other oxygen-utiliz-



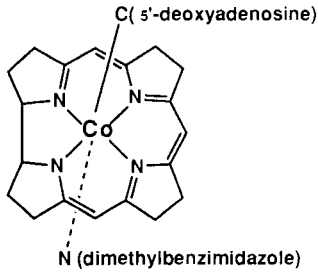
Hemin: Fe-protoporphyrin IX chloride

7

ing systems (*39*). The exact mechanism for this is unclear, but regulation of the transcription of yeast cytochrome *c* genes involves a

heme-activating protein with a DNA-binding domain containing Zn–Cys fingers and a heme-binding region. There is clearly much scope for new drugs aimed at modifying the biosynthesis and breakdown of hemin in cells.

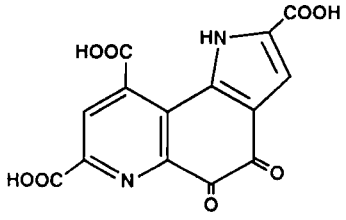
The related cobalt cofactor vitamin B_{12} (**8**), the coenzyme of methylmalonyl CoA mutase and homocysteine transmethylase, contains a



Vitamin B_{12} (core structure)

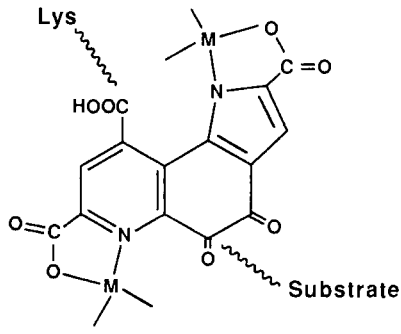
8

corrin ring. It cannot be synthesized by mammalian cells and must be taken in as a dietary component. Another metal-binding cofactor for which there appears to be a high dependence on nutrition, or on intestinal flora (*40*), is pyrroloquinoline-quinone (PQQ, **9**). It is a cofactor for



Pyrroloquinoline-quinone (PQQ)

9a



Metal-protein binding

9b

copper enzymes such as amine (e.g., lysyl) oxidases and iron enzymes such as lipoxygenase and adrenal medulla dopamine β-hydroxylase (*41*). Again, synthetic analogs of this cofactor may prove to be useful as regulators of metabolic biochemistry.

F. Antiperspirants

Both Al(III) (ionic radius 0.50 Å) and Zr(IV) (0.72 Å) are small, highly charged and highly polarizing ions. They form strong oxygen-bridged polymers (hydroxide, oxide, and carboxylate) that break down very slowly. This is the basis of their use as antiperspirants and deodorants for coating the skin. It is important that the complexes remain intact and that Al(III) especially is not absorbed into the bloodstream (see Section IV,A). Zirconium aluminium glycine complexes and aluminium chlorhydrate, both widely used in antiperspirants, can produce allergic reactions when injected intradermally into animals. A few other metal salts are also established allergic sensitizing agents, including $K_2Cr_2O_7$, BeF_2, $HgCl_2$, and platinum chlorides (vide infra) (*3*). Indeed, eczema due to contact with cement, and the trace levels of $K_2Cr_2O_7$ that it contains, is a very frequent occupational hazard (*42*). The chemical basis for these potent immunological effects may involve the formation of antigenic metal–protein complexes, but this requires further investigation.

III. Active Elements

A. Lithium Drugs

Li^+ is a highly labile metal ion, with water exchange rates of $\sim 10^9$ sec^{-1}. It binds only weakly to ligands, showing a preference for oxygen. It is a very small ion (ionic radius 0.6 Å), but with its high charge density it is strongly solvated. In Britain, about 1 in every 1500 people take gram quantities of Li_2CO_3 daily for the treatment of manic depression (*43*). Spa waters are often rich in lithium, but the discovery of its use in controlling mood came by accident when Cade experimented with lithium urate because it was a soluble urate salt.

On account of their similar sizes, Li^+ may interfere with the natural biochemistry of Mg^{2+} (ionic radius 0.65 Å), which is required to activate several intracellular enzymes, especially those which bind ATP. But lithium may also affect calcium mobilization in cells by inhibiting enzymes in the inositol phosphate pathways. This may slow down the supply of lipid precursors required to generate second messengers such as inositol-1,4,5-triphosphate, which in turn regulates the mobilization of Ca^{2+} from intracellular stores and its entry into cells. Slow-release lithium drugs would be useful in the clinic, with such designs being possibly based on caged ligands or sparingly soluble minerals. High concentrations of lithium (5–40 *mM*) inhibit the replication

of DNA viruses such as herpes, pox, and adenovirus, but not RNA viruses (*44*).

B. Gold Antiarthritic Drugs

All the gold compounds used in therapy today (chrysotherapy) are Au(I) compounds (*45–47*) (**10–14**). Gold has an intriguing chemistry dominated by relativistic effects (*48*) and it is interesting to consider the reasons why Au(III) complexes are not used as anticancer agents like those of Pt(II) are used.

Gold(III) is isoelectronic and its complexes are isostructural (square–planar) with those of Pt(II). However, ligand substitution reactions occur much more rapidly than do those of Pt(II), and *cis*-diamminodihalogold(III) analogs of cisplatin have not yet been characterized. Reactions of $[Au(NH_3)_3(Hal)]^{2+}$ and $[Au(NH_3)_4]^{3+}$ with halide give only the trans product. The rates of cyanide exchange reactions for tetracyano complexes of d^8 metal ions illustrate the high lability of Au(III) (Fig. 2) (*48a*). Gold(III) complexes are also significantly stronger oxidants than Pt(II) complexes, and redox reactions often compete with ligand substitution reactions. Gold(III) is also a very soft metal ion (*49*), showing a high discrimination between ligands (order of stability constants in water):

$$SCN^- > CN^- > NH_3 > OH^- \gg Br^- > I^- > H_2O$$

The acidity of amines is enhanced by more than 30 orders of magnitude when coordinated to Au(III); for example,

$$[Au(NH_3)_4]^{3+} \rightleftharpoons [Au(NH_3)_3(NH_2)]^{2+} + H^+ \qquad (pK = 7.5)$$

Aqua complexes of Au(III) are also highly acidic; $[AuCl_3(H_2O)]$ has a pK_a of 0.6 (*50*), although hydrolysis reactions sometimes occur relatively slowly. Simple halide ions such as $[AuCl_4]^-$ are too strongly oxidizing to be used as drugs and will oxidize methionine to the sulfoxide, cystine disulfides to sulfonates, and carboxylates to CO_2. Gold(III) is stabilized with respect to Au(I) and Au(0) by N-donor ligands, and Au(III) porphyrins are difficult to reduce.

Metallic gold has a very high thermodynamic stability, and the use of red or purple colloidal gold solutions (gold sols, aurum potabile) in medicine was popular during the Renaissance period. The colors depend on the sizes of the particles and can be controlled in reactions of $[AuCl_4]^-$ with appropriate reducing agents such as citrate. The particles carry a high negative charge and proteins are strongly adsorbed, forming the basis of a method widely used today for probing antibody

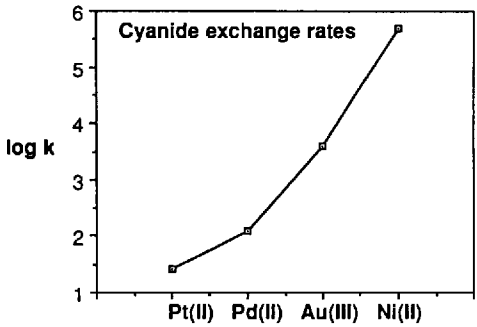
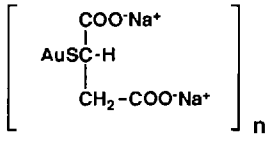


FIG. 2. Second-order rate constants for the exchange of cyanide with $[M(CN)_4]^{x-}$ (M = d^8 metal ion) complexes, showing the high kinetic lability of Au(III) relative to Pt(II). (Data from Ref. *48a*.)

sites on cells by electron microscopy. Colloidal gold particles are phagocytosed (eaten) by cells and stored in vesicles, and so are not therapeutically active, although radiolabeled particles can be useful for imaging the liver (^{198}Au), or destroying local joint tissue.

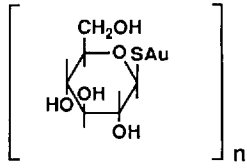
Neither Au(I) nor Au(III) aqua ions, $[Au(H_2O)_2]^+$ and $[Au(H_2O)_4]^{3+}$, have been characterized in solution or the solid state. The diaqua Au(I) ion is unstable to disproportionation, and stabilization of Au(I) requires π-acceptor ligands. The dicyano complex $K[Au(CN)_2]$, with the characteristic linear two-coordination found in the majority of Au(I) complexes, is very stable and was used with some success to treat tuberculosis at the beginning of the century. Toxic side effects were reduced with thiolate complexes such as aurothiomalate (**10**), and the French physician Forestier, in 1929, introduced gold(I) thiolates for the treatment of rheumatoid arthritis in the mistaken belief that the two diseases were related (*51*).

A variety of injectable gold(I) thiolates (**10–13**) are now used to treat difficult cases of rheumatoid arthritis and are given in doses of about 25 mg per week for several years. Beneficial effects are often not seen



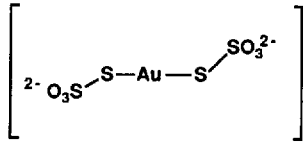
Aurothiomalate (Myocrisin)

10



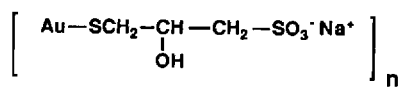
Aurothioglucose (Solganol)

11



Auro-bis(thiosulfate (Sanochrysin)

12



Aurothiopropanolsulfonate (Allochrysine)

13

for several months; several grams of gold are given during the course of treatment, and gold can remain in the body for many years after treatment has stopped. Blood concentrations of gold can reach 40 μM, and careful monitoring is required to avoid toxic effects, e.g., on the kidney. When used carefully, gold drugs can succeed in halting joint erosion when organic drugs fail.

These 1 : 1 Au(I) thiolate antiarthritic complexes have not been crystallized, but X-ray absorption (*52*), X-ray scattering (*53, 54*), NMR, and Mössbauer spectroscopy (*55, 56*), as well as gel permeation chromatography, show that they are not simple monomers but are composed of ring and chain structures (Fig. 3). Commercial drugs often contain a slight molar excess (up to 15%) of thiol over gold. The dissolution behavior of aurothiomalate is curious: initially, aqueous solutions are yellow (two bands at 337 and 370 nm), but decolorize over a period of ~1 hr, a process that can be reversed by increasing the ionic strength (*57*). These changes can be attributed to changes in polymer conformation, and in Au–Au contact distances. Short Au–Au contacts are remarkable features in the crystal structures of many Au(I) complexes (*58*), often effectively increasing the coordination number of Au(I) to four; even the triply charged anions $[Au(S_2O_3)_2]^{3-}$ (**12**) form pairs in the crystal lattice of the sodium salt, with Au–Au contacts of 3.24 Å (*59*). These contacts may stabilize gold(I) thiolate polymers, because the equilibrium constants for the breakdown of polymers to form monomers

$$1/n[AuSR]_n + RSH \rightleftharpoons [Au(SR)_2]^- + H^+$$

($\sim 10^4\ M^{-1}$) are not as high as might be expected, and the reaction is readily reversed to the left at acid pH values. Neither tris- nor tetrakis-thiolato gold(I) complexes have been characterized, in contrast to the isoelectronic Hg(II). It is notable that the compartments of cells that accumulate much gold (lysosomes) can be quite acidic (pH 5) and that bisthiolato gold(I) complexes readily revert to 1 : 1 polymers at low

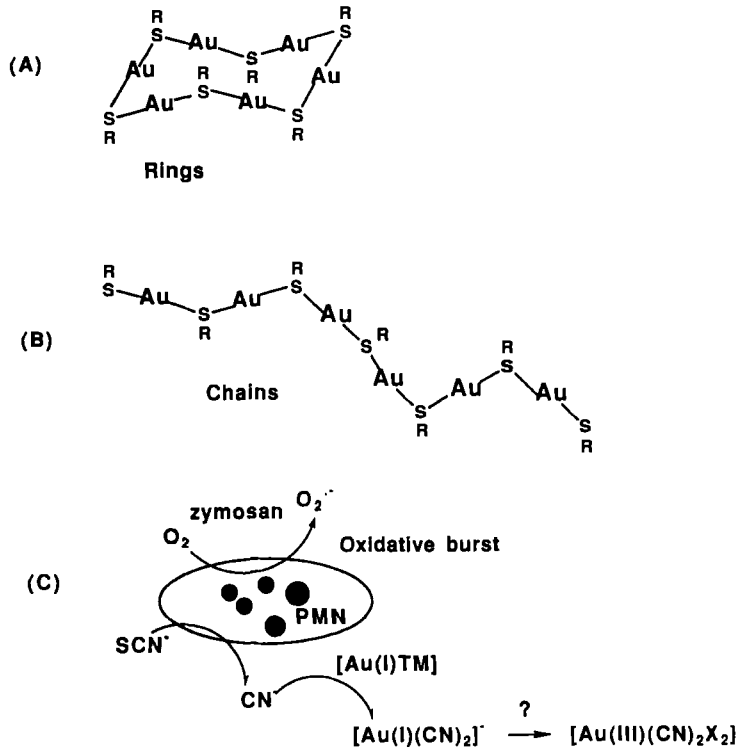


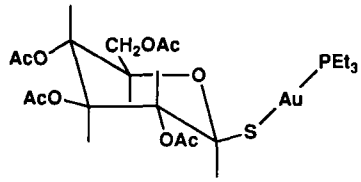
FIG. 3. (A) Ring and (B) chain structures for gold(I) thiolate drugs such as aurothiomalate ("Myocrisin"). Wide-angle X-ray scattering measurements have revealed Au–Au contacts of 3.35, 5.8, and 8.1 Å, and analysis of the fine structure on the Au L_{III} X-ray absorption edge gives an average Au–S distance of 2.3 Å with a coordination number of two (sulfurs) (*54*, *52*). (C) White blood cells (PMNs) can covert aurothiomalate into $[Au(CN)_2]^-$, an inhibitor of $O_2^{\cdot-}$ production (the oxidative burst) (*62*). This could provide a route to Au(III) dicyano complexes.

pH (*60*). Other potential metal-binding ligands available *in vivo,* such as Met sulfur, His nitrogen, or Asp and Glu carboxylates, are not able to compete with thiolates either in monomers or polymers; indeed, Au(I) bonds to oxygen are very weak and rare.

Preferred binding sites for gold(I) are thiols with low pK_a values (*60*) and thiol exchange reactions are usually rapid. Thus, *in vivo,* over 80% of circulating Au(I) from aurothiomalate is carried on albumin at Cys 34 (*61*), which has an estimated pK_a of <5, initially as albumin–Cys34–S–Au(thiomalate), and perhaps even as albumin–Cys34–S–$\{Au(thiomalate)\}_n$. But the thiol ligand in the original drug, e.g., thiomalate, is readily displaced and metabolized independently of gold.

Shaw has suggested that the active circulating species is albumin–Cys34–S–Au(glutathione). Much more needs to be known about the mechanisms of gold transfer from albumin into cells. An intriguing possibility is that the process is mediated by cyanide, as suggested by Graham and co-workers. Smokers inhale thiocyanate, and have elevated plasma thiocyanate levels (50–200 μM) compared to nonsmokers (~40 μM). Their red cell gold levels are much higher than nonsmokers; SCN^- is converted into CN^- (HCN at pH 7) by polymorphonuclear leukocytes (PMNs, white blood cells). It can be shown that PMNs can convert aurothiomalate (and albumin-bound gold) into $[Au(CN)_2]^-$ (a potent inhibitor of O_2^- production by PMNs) *in vitro* (*62*). Cyanide readily displaces thiomalate from Au(I) (*63*) and these reactions may play a role in the metabolism of gold in both nonsmokers and smokers. It is notable that Au(III) dicyano complexes have greatly enhanced thermodynamic stabilities (*49*) and it is therefore possible that Au(III) plays a role in the metabolism of gold *in vivo* (Fig. 3), although this has not yet been demonstrated. Perhaps cyanide also plays a role in metabolism of other metals such as platinum, iron, and copper.

The orally active Au(I) complex auranofin (**14**) has a well-defined, linear two-coordinate structure (*64*). It is a lipophilic complex, but



Auranofin

14

hydrolysis of the acetyl groups probably occurs during absorption in the intestine (*65*) and so the species that enters the blood may be Au(I)(PEt_3)(β-D-thioglucose). The Au(I)–phosphine bond is strong, but thiol exchange reactions are facile, giving albumin–Cys34–Au–PEt_3, readily identified by its ^{31}P NMR spectrum (*66*). Further slow reaction with thiols leads to release and oxidation of the phosphine to $OPEt_3$ and consequently the end products of the metabolism can be the same as those of injected gold(I) thiolate drugs. Again, the ligands and gold(I) are metabolized and excreted at different rates.

In vivo, much of the administered gold ends up in membrane-bound compartments of cells called lysosomes ("aurosomes"). These contain

proteolytic enzymes that can destroy joint tissues. EXAFS measurements have suggested that gold in aurosomes is a two-coordinate gold(I) dithiolate complex, but its identity is unknown. It could be a protein complex, perhaps with the thiol-rich (20 Cys out of 61 amino acids) protein metallothionein (MT). Gold(I) from aurothiomalate can displace both Zn(II) and Cd(II) from metallothionein, in the limit, giving Au_{20}MT. However, auranofin does not react with Zn,Cd-MT, although it does react with apo-MT (*61*). In this way, Au(I) may interfere with the metabolism of Cu(I), which is stored as metallothionein in cells and may be cycled through lysosomes in which copper is mobilized. Lysosomes can have low pH values and these may favor Cu(I) dissociation from thiols and perhaps oxidation to Cu(II), but the high affinity of Au(I) for thiols even at low pH and the difficulty of oxidation may be responsible for the long-term retention of gold in lysosomes. The inhibition of the metalloenzyme collagenase by Au(I) will be discussed in Section IV,E. Gold(I) attack on steroid receptor proteins could provide feedback into both Zn(II) and hormone metabolism. So-called hormone-responsive DNA sequences are regulated by steroid-binding proteins, which bind to steroids in the cell cytoplasm, change conformation, and subsequently bind to DNA. The hormone-receptor proteins are known to contain Zn(II)-binding sites, including Cys-rich zinc-finger motifs, typically (*67*):

X-X-Cys-Tyr-X-Cys-Gly-X-X-Gly-His-X-Ala-X-X-Cys-X-X-

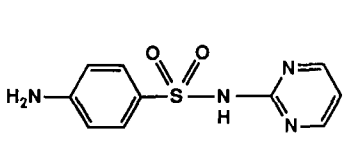
Because these are likely to be in flexible loop regions, they may be accessible to Au(I) and provide a mechanism by which Au(I) can interfere with steroid metabolism.

New developments associated with gold(I) antiarthritic drugs are taking place in two areas. First, some Au(I) thiolate complexes, such as Au(I) thioglucose (AuTG), have been found to be potent inhibitors of human immunodeficiency virus (HIV) reverse transcriptase (*68*). Inhibition occurs at concentrations as low as 3 μM AuTG, and the gold complex appears to block access of the enzyme to the RNA template. Complexes related to gold thioglucose may therefore be useful as antiviral agents, if a way can be found to transport them into cells. Second, auranofin is highly cytotoxic to tumor cells in culture, which raises the possibility of its use as an anticancer drug (*69*). This is discussed further in Section IV,C. It should be noted, however, that the high *in vitro* cytotoxicity of auranofin is greatly modulated *in vivo*. If *in vitro* cell testing had been the primary screen, the compound would never have reached the clinic as an antiarthritic agent. In general, primary

screening of metal complexes using cell cultures has to be carried out with great care because a large number of complexes may be toxic at relatively low doses.

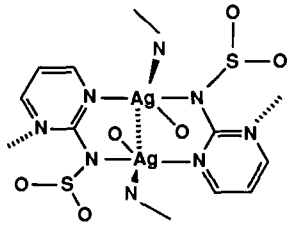
C. Silver and Mercury Antimicrobial Agents

Many Ag(I) compounds are potent antibacterial agents, although the reasons for this are not well understood. Silver sulfadiazine (**15**) is



Sulfadiazine

15a



15b

widely used in the clinic. It is applied as a topical cream for the treatment of severe burn wounds for which infection by *Pseudomonas putida* can be fatal. It is an insoluble, polymeric complex that acts as a slow release agent for Ag(I) ions. Electrolytic production of Ag(I) ions from nylon cloth impregnated with metallic Ag (3 g per m^2) is also very effective. Although bacterial resistance to organic antibiotics is common, it is said (A. T. McManus, personal communication) that bacterial resistance to Ag(I) ions has never been demonstrated. Reported cases appear to be ones of sulfadiazine resistance, not Ag(I) resistance.

Some mercury complexes are still used as antimicrobial agents, although bacterial resistance to Hg(II) is common and is often associated with extrachromosomal DNA (plasmids) (*70*). It is now well understood at the molecular level. A series of genes code for proteins that detoxify Hg(II) by capturing it at the bacterial cell wall, transporting it to the cytoplasm, and reducing it to volatile Hg(0). Mercuric reductase contains a redox-active Cys—Cys disulfide bond: Cys-X-X-X-X-Cys, where X is an amino acid other than Cys. A key feature of the enzyme appears to be its ability to impose unusual stereochemistry on Hg(II) [three or four coordination using Cys thiols (*71*)], perhaps to control the kinetics of mercury uptake and release, and its redox potential—an example of an *entatic state* (*72*). The metalloregulatory protein merR switches from being a repressor to a transcriptional activator upon binding Hg(II), which it does with an affinity 10 times higher than that of model Hg(II) bisthiolate complexes (*73*). Again, three or

four thiolates are thought to be involved in Hg(II) binding to the protein. Au(I) is likely to be an inhibitor of mercuric reductase because it does not readily form three- and four-coordinate thiolato complexes; however, it would be interesting to investigate the redox potentials for Au(I) in such an unusual site. It would also be interesting to explore the chemistry of Hg(0) in this system and the association of Hg(0) atoms into clusters.

D. Control of Mineralization

Mineralization in the body is a carefully controlled process; if errors occur, then serious conditions can result (Table VII). Hydroxyapatite in bone not only acts as a hard structure but also as a store of elements, and is continuously being broken down and remodeled. In diseases such as Paget's disease, in which there is excessive bone turnover, and in neoplastic disorders of skeletal metabolism, it is possible to gain some control of mineralization with pyrophosphate analogs such as dichloromethylene diphosphonate (the drug clodronate), which inhibits the activity of osteoclasts, the cells that remodel bone. It is notable that phosphonate ligands can be used to target radioisotopes to bone for diagnostic imaging (Section III,E).

TABLE VII

Minerals in the Body

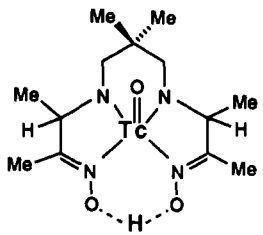
Mineral	Function or condition
Calcium carbonate	
$CaCO_3$ (aragonite)	Gravity device—inner ear (organic composite)
Calcium phosphate	
$Ca_{10}(PO_4)_6(OH)_2$ (hydroxyapatite) (plus F^-, Cl^-, Mg^{2+}, and Na^+)	Hard tissues, tooth enamel, bones, urinary calculi
$Ca_8H_2(PO_4)_6$ (octacalcium phosphate)	Dental calculi, bones, renal calculi
$Ca(HPO_4) \cdot 2H_2O$ (Brushite, soluble)	Bones, teeth, renal stone formation
β-$Ca_3(PO_4)_2$ (Whitlockite, plus Mg and Fe)	Renal stones, dental calculi
$Ca_2P_2O_7 \cdot 2H_2O$ (pyrophosphate)	Pseudogout (crystals in knee joints)
Calcium oxalate	
$CaC_2O_4 \cdot H_2O$ (Whewellite)	Urinary stones
$CaC_2O_4 \cdot (2 + x) \cdot H_2O$ (Weddelite)	
Iron oxide	
$5Fe_2O_3 \cdot 9H_2O/PO_4^{3-}$ (ferrihydrite)	Iron store (ferritin)

In the mouth, mineralization is controlled partly by proteins such as statherin from the salivary gland, which maintains a supersaturation of calcium and phosphate in the mouth. The basic mineral in tooth enamel is hydroxyapatite, in which it is possible to substitute Sr(II) for Ca(II), and F^- for OH^- isomorphously, hence the use of strontium and fluoride salts in toothpastes, said to confer extra strength to the enamel and antibacterial properties.

Mineralization in apoferritin involves a prior oxidation of Fe(II) as it enters channels in the protein shell. The ferroxidase center seems to be composed of Glu (Gln) and His residues situated between four helices (P. M. Harrison, personal communication). There is scope for exploring the design of agents that could block the entry of iron into the core of the protein or hasten its passage out. It is possible that non-redox-active metal ions such as Ga(III), In(III), and Al(III) can act in this way. The nature of the Fe(II) complex in the cytoplasm, which acts as a donor to ferritin, is not clear yet, but perhaps it could be Fe(II) glutathione.

E. Radiopharmaceuticals

Radiopharmaceutical agents are chosen largely because of the favorable energies of emission and time scale of decay of certain isotopes (*2, 3, 4, 74*) (Table VIII). The chemical compositions of many of the agents used have not been fully elucidated, on account of the low concentrations of metal ions in the preparations and often the need to generate the complex immediately before administration. However, this situation is changing. For example, the new ^{99m}Tc(V)–HMPAO complex (**16**) for assessing regional blood flow in the brain has a well-defined



16

structure, as do the octahedral, positively charged Tc(I) hexakisisonitrile complexes used for the investigation of cardiac perfusion (*75*). Further understanding is required of the mechanisms by which these

TABLE VIII

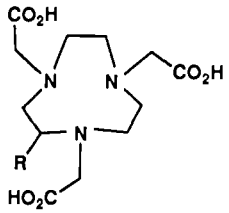
EXAMPLES OF THE USE OF RADIOISOTOPES IN MEDICAL DIAGNOSIS AND IMAGING[a]

Isotope	Decay	Compound	Use
^{51}Cr	27.7 days (K, γ)	Cr(III)(EDTA)	Glomerular filtration rate
		Cr–albumin	Placental localization
		Cr–red cells	Blood loss, spleen imaging
^{64}Cu	12.7 hr (β^+, β^-, γ)	CuEDTA (acetate)	Diagnostic brain scan (Wilson's disease)
^{57}Co	271.8 days (K, γ)	Vitamin B_{12}	B_{12} tracing
^{67}Ga	3.3 days (K, γ)	Ga(III) citrate	Neoplastic, inflammatory lesions
^{99m}Tc	6.0 hr (IT, γ)	Tc(EDTA)	Glomerular filtration rate, renal imaging
		Tc–albumin	Blood pool imaging
		Tc–polyphosphonates	Bone, myocardial imaging
		Tc–HMPAO	Cerebral perfusion imaging
^{111}In	2.8 days (K, γ)	In(III)–bleomycin	Tumor detection
		In(III)–oxine	Labeled leukocytes (abscesses, infections, inflammations), labeled platelets (thrombosis, transplant rejection)
^{198}Au	2.7 days (β^-, γ)	Colloidal Au(0)	Liver imaging
^{201}Tl	3.0 days (K, γ)	Tl(I)Cl	Diagnostic myocardial imaging

[a] See Refs. *3* and *74*.

complexes become localized and trapped in particular tissues and cells. In the case of ^{99m}Tc–HMPAO, this may be dependent on the small change in pH across the blood–brain barrier and change in ionization state of the ligand. Little is known about redox reactions of Tc complexes inside cells.

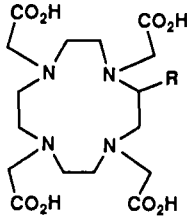
The derivatization of macromolecules with ^{99m}Tc and other radioisotopes should aid the targeting of radioisotopes for both diagnostic imaging and therapy. For imaging, for example, monoclonal antibodies can be derivatized with metallothionein and then labeled with up to seven TcO^{3+} ions with square–pyramidal geometry (*76*). Although Zn(II) dissociates from the protein at pH 4, the Tc ions remain firmly bound. Another promising approach involves labeling with triazacyclododecane and triazacyclononane macrocycles (*77–79*). The ^{111}In complex of (**17**) is cleared from the tissues of mice within a few hours of injection, whereas the modified EDTA and DTPA complexes lead to ^{111}In transfer to the plasma (Fe-binding) protein transferrin and deposition in the liver. The remarkable kinetic inertness of complexes of



R = H : NOTA

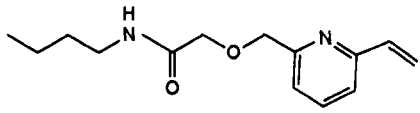
17

these tri- and tetraaza macrocycles is exemplified by the stability of the ^{67}Ga complex of **17**, which is resistant to dissociation in 1 *M* acid for >2 weeks. The rates of metal association are limited by the rates of proton displacement and can also be very slow. Efficient labeling of the macrocycle with the radioisotope is best achieved before antibody conjugation. For therapy, labeling with destructive isotopes such as ^{90}Y or ^{203}Pb as a source of cytotoxic daughter ^{212}Bi is possible. The Y complex of **18** is very stable and is rapidly cleared from the body. Analogous



DOTA
Y ligand

R = H : DOTA
R =



for attachment to Cys SH of antibody

18

kinetically inert complexes with Gd(III) are effective relaxants for water (*80*) and potentially useful contrast agents for NMR imaging (Section II,D).

An interesting proposal for cancer therapy using ^{57}Fe has been made by Mills *et al.* (*81*). They have claimed that delivery of ^{57}Fe–bleomycin to tumor cells followed by photoactivation with 14.4-eV resonant Mössbauer γ rays can produce tumor regression at doses as low as 10^{-5} Gy. DNA damage appears to be caused by emission of Auger electrons, which leads to a cluster of positively charged ions and a "molecular explosion." It remains to be seen whether this technique is useful only

for surface tumors, and whether it can be carried out with other Mössbauer isotopes.

F. Boron for Neutron Capture Therapy

Nuclides with neutron capture cross-sections high enough to make them potentially useful in therapy include ^{6}Li, ^{10}B, ^{113}Cd, ^{149}Sm, ^{151}Eu, ^{155}Gd, ^{157}Gd, and ^{174}Hf. The properties of ^{10}B are favorable, and boron compounds have been tested clinically (for over 20 years in Japan) for the treatment of melanomas, bone marrow and malignant brain tumors (*82*). The isotope ^{10}B undergoes fission on bombardment with thermal or epithermal neutrons, giving rise to α particles that are destructive to local tissue (within 10 μm):

$$^{10}_{5}B + {}^{1}_{0}n \rightarrow {}^{7}_{3}Li + {}^{4}_{2}He + 2.7\ MeV$$

Administered boron compounds need to be selective for tumor tissue so that damage to normal tissue is minimized, and about 10^9 ^{10}B atoms are required for the destruction of a tumor cell (J. H. Morris, personal communication). Early trials were carried out with *p*-carboxybenzeneboronic acid and sodium dodecahydrododecaborate ($[B_{12}H_{12}]^{2-}$). Although these are relatively nontoxic, they are nonselectively distributed. More promising is the monomercapto derivative of $[B_{12}H_{12}]^{2-}$, $[B_{12}H_{11}(SH)]^{2-}$ (**19**), which, although more toxic, shows

$[B_{12}H_{11}SH]^{2-}$

19

greater selectivity in tumor uptake (*83*). This polyhedral borane anion is water soluble and stable and shows a high affinity for proteins, perhaps forming disulfide linkages. It might be possible to deliver boron using antibodies, but each antibody molecule will have to carry $\sim 10^3$ ^{10}B atoms. There is scope for further investigations of the biological chemistry of borane clusters and for further chemical modification of their properties.

Only two naturally occurring boron compounds are known, both antibiotics: aplastomycin from *Streptomyces griseus* and boromycin from *Streptomyces antibioticus*. Boron has been known to be essential for plant growth since 1923, but only in 1981 was it recognized to be important to mammals (*6*). It plays a role in Ca and P metabolism, preventing calcium loss and demineralization of bones. Nielsen believes that boron is a significant nutritional factor in preventing osteoporosis, and has postulated a role for boron in steroid hydroxylation. The daily requirement is probably ~0.2 mg, and is mainly met from fruit, nuts, and vegetables (and wine!). Boronic acids, e.g., BOC-Ala-Pro-boro-Val-OH, are potent inhibitors of serine proteases.

IV. Active Ligands

A. Antacids

Relatively insoluble salts of alkaline earth metals such as Ca(II) carbonate, Mg(II) carbonate, hydroxide, trisilicate, and oxide provide a slow release of base to neutralize acidity in the stomach. Absorption of the cation is safe, although not without side effects: Mg(II) is one of the few cations that can act as a laxative, and $MgSO_4$ is widely administered for this purpose. Little modern research appears to be directed toward redesigning these agents, but work involving organic composites or doped lattices might be profitable. Al(III) and Bi(III) salts are also marketed as antacids, as, e.g., the hydroxide, and citrate, subnitrate, and carbonate, respectively. The pK_a of water coordinated to Al(III) is lowered to ~4, and for Bi(III) to ~2, and therefore these ions themselves offer buffering capacity at an acid pH. They must be used with care in pharmacology because absorption of Al(III) into the bloodstream can lead to dementia. Al(III) is carried by the Fe(III)-binding protein transferrin and is deposited in the brain as aluminosilicate plaques. Absorption of Al(III) is aided by citrate in the diet (e.g., from fruit) and is retarded by silicates. Indeed, it has been suggested (*7*) that Si is an essential element merely to protect against Al toxicity. The Fe(III) chelating agent desferrioxamine is currently used to remove Al(III) from the body, but more work is required to determine how to remove Al from the brain.

Bismuth nitrate ("magisterium bismuti"), a white pigment, was used in beauty care, painting, and as a medicant in the seventeenth century, and staggering amounts of bismuth compounds have been administered for therapeutic purposes in the past, for example, over a

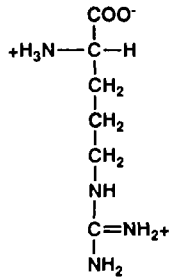
quarter of a ton was given to patients on the Gold Coast of Africa from 1933 to 1942 (*84*). The hydrolysis of bismuth nitrate begins at pH values as low as 1, with the formation of clusters such as $[Bi_6O_4(OH)_4](NO_3)_6$ (*85*). In this century, various bismuth salts have been used to treat syphilis, as antihypertensives, as diuretics, and for the treatment of gastrointestinal disorders. Today, colloidal bismuth(III) subcitrate is available for the treatment of peptic ulcers (for which it can be as effective as organic drugs such as cimetidine), and bismuth subgallate and oxide are used as skin antiseptics (*86*). However, an outbreak of bismuth encephalopathy in France in the 1970s led to withdrawal of oral bismuth drugs from the market there.

A striking feature of the cellular effects of bismuth compounds in animals (and one shared only by lead) is the production of intranuclear inclusion bodies of up to 5 μm in diameter (*87*), for example, in the tubular epithelial cells of the kidney. Electron probe microanalysis shows that these contain both Bi and S, and so could be a complex with a Cys-rich protein such as metallothionein. Bismuth is known to be a potent inducer of renal metallothionein synthesis, and pretreatment of animals with bismuth salts can prevent some of the toxic side effects induced by cisplatin (*88*). The role of metallothionein in the pharmacology of bismuth remains to be established, but the strong involvement of zinc, also an inducer of metallothionein synthesis, in the metabolism of skin cells, for example, may be related. Like several other elements of Group V, the development of the biological chemistry of Bi is hampered by the lack of good physical properties, in particular of a well-behaved NMR isotope.

B. Antihypertensive Agents: Nitric Oxide as a Muscle Relaxant

Nitrous oxide, N_2O, laughing gas, has long been known to have anesthetic properties, but the activity of nitric oxide, NO, as a muscle relaxant has been elucidated only in the last few years. The so-called endothelium-derived relaxing factor (EDRF) is now thought to be NO, and is a product of L-arginine (**20**) oxidation in the body (*89*). Nitric oxide is a potent activator of the heme and copper-containing enzyme guanylate cyclase, probably by binding directly to iron and possibly copper. The half-life of the reactive, paramagnetic NO in tissues is only 6 sec, and delivery systems are thought to include sodium nitroprusside, sodium azide, nitroglycerin, hydroxylamine, and sodium nitrite, all of which can cause smooth muscle relaxation.

Sodium nitroprusside, $Na_2[Fe(CN)_5NO]$ (**21**), is a potent hypotensive agent widely used to control blood flow after myocardial infarction and



Arginine

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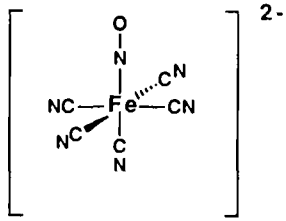

Nitroprusside

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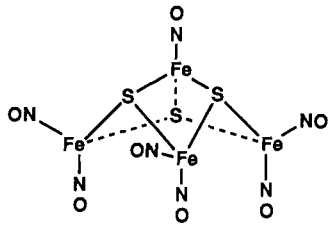
during surgery (*90*). It is a low-spin diamagnetic, octahedral Fe(II)d^6 complex with an almost linear Fe—N—O (formally NO^+) linkage with Fe—N and N—O bond lengths of 1.63 and 1.13 Å, respectively (*91*). A typical infusion dose is 3 μg/kg/min. The compound is photoreactive and must be protected from light: a 0.67 m*M* solution decomposes by ~50% on standing 2 hr in the sunlight (*92*). The NO ligand is essential for activity; $Fe(CN)_5L$ complexes with L = NO^+, NO_2^-, and NOS^- are active, but those with L = H_2O, NH_3, and CN^- are inactive in lowering blood pressure (*93*). The manganese analog $[Mn(CN)_5NO]^{3-}$ is also inactive, perhaps because it contains a neutral NO ligand, which is not easily displaced, e.g., by thiols. Nucleophilic attack of thiols on coordinated NO^+ may be important in the mechanism of action. The reaction of cysteine with nitroprusside at pH 7.5–11 gives an intense red coloration, thought to be due to $[Fe(CN)_5NO(S\text{-}Cys)]^{3-}$, followed by a catalytic reaction in which cysteine is oxidized to cystine (*94*), but the chemistry of nitrosothiols is poorly understood. It has recently been suggested that the natural muscle-relaxing factor (EDRF) is a nitrosothiol such as nitrosocysteine rather than NO itself (*95*).

Nitric oxide may play a role in regulating the cardiovascular system, in platelet function, in renin release, in mesanglial cell activity, and in other systems. Further understanding of the arginine:NO pathway may provide novel approaches to the design of new pharmaceutical agents. Other products of arginine oxidation are nitrate and nitrite, which are both synthesized by a class of white blood cells, macrophages (*96*). Clearly, if metal nitrate salts are tested for biological activity, then the anion itself may exert significant effects. It is apparent that the natural biochemistry extends all the way from −3 to +5 oxidation states, and it is likely that a wide range of metalloenzymes is responsible for their interconversion, as in the case of bacteria [Fe, Cu (*97*)].

Perhaps there are also enzymes that catalyze the formation and breakdown of N—N bonds in man.

Nitric oxide complexes of iron–sulfur clusters have interesting biological properties. $[Fe_4S_3(NO)_7]^-$ (**22**), a product of autoclaving nitrite,



$[Fe_4S_3(NO)_7]^-$

22

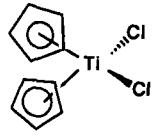
cysteine, and Fe(II), is an antimicrobial agent and disinfectant; in contrast, the microbial product $[Fe_2(SMe)_4(NO)_4]$ formed in nitrate-rich waters in China is a suspected contributor to esophageal cancer (*98*).

C. Anticancer Agents, Radiosensitizers, and Antiviral Agents

1. Anticancer Agents

It is possible to incorporate reactive ligands into platinum anticancer agents to improve their targeting, for example, DNA-intercalating groups (polycyclic aromatics), alkylating agents, or antimetabolites (modified nucleosides or sugars) (*15, 99*).

Metallocenes are examples of metal complexes that have little activity in the normal first-line screens for anticancer activity: P388 and L1210 leukemias. In contrast, titanocene (**23**), vanadocene complexes



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($[(C_5H_5)_2M(IV)X_2]$), and ferrocinium complexes ($[(C_5H_5)_2Fe(III)]^+$) exhibit systemic activity against numerous other experimental tumors (Table IX) (*100, 101*). Examples are the red complex $(C_5H_5)_2Ti(IV)Cl_2$ and its green V(IV) analog. Titanocene complexes are readily hydro-

TABLE IX

ANTITUMOR AND OTHER BIOLOGICAL ACTIVITIES OF METAL CYCLOPENTADIENYL COMPLEXES[a]

Active complexes

1. $(C_5H_5)_2MX_2$
   M = Ti(IV), V(V) > Nb(IV), Mo(IV) ≫ Ta, W, Zr, Hf
   X = e.g., halide, pseudohalide
2. $[(C_5H_5)_2M]X$
   M = e.g., Fe(III); X as above
3. $(C_5R_5)_2M$
   M = Sn(II), Ge(II); R = e.g., Ph

Antitumor activity

Ehrlich ascites, sarcoma 180, B16 melanoma, colon 38 carcinoma, Lewis lung carcinoma, human carcinomas in xenographs (*inactive*: L1210 and P388 leukamias)

Ligands

Cyclopentadiene and dicyclopentadiene are local tumor inhibitors at high doses, no systemic activity

Mechanism and distribution

Inhibition of DNA synthesis; Ti and V are found in nucleotide-rich areas of cells and increase mitotic activity and induce virus particles; facile loss of C_5H_5 ligands; Ti distribution: liver and intestines (low in kidneys and lungs)

Antiviral and antiinflammatory activity

Titanocene complexes

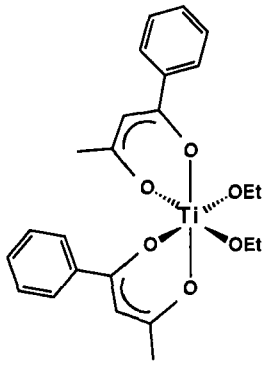
[a] Refs. *100* and *101*.

lyzed in aqueous media (*102*) and it seems likely that titanium is acting partly as a carrier for the highly reactive cyclopentadiene ligand, enabling it to reach intracellular target sites not accessible to cyclopentadiene alone (*101*). Indeed, both cyclopentadiene and its Diels–Alder dimer, which is the room-temperature-stable form, are active with cure rates comparable to the η^5-cyclopentadienyl metal complexes [101], but only to local tumors, and then only at high doses.

Acetylacetonate complexes such as budotitane (**24**), now in phase 2 clinical trials for the treatment of colon cancer (*103*), may also act as ligand delivery systems. Budotitane, $[Ti(IV)(bzac_2)(OEt)_2]$, where bzac is the anion of 1,3-diphenyl-1,3-propanedione, is administered at doses of 14–21 mg/kg (total dose 0.5–2 g), and serum Ti levels range from 40 to 250 μM. For M(IV) complexes the activity order (sarcoma 180) is

$$Ti(IV) \sim Zr(IV) > Hf(IV) > Mo(IV) > Sn(IV) > Ge(IV) \text{ (inactive)}$$

There are five possible geometrical isomers of budotitane, but the relative contributions of each of these to the activity is not known and may be difficult to determine because the three isomers of the thermodynamically more stable cis complex undergo rapid intramolecular



Budotitane

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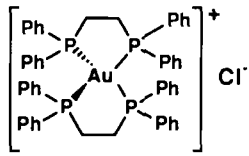
interconversion in dichloromethane (*104*). The complex is readily hydrolyzed to TiO_2 via aqua, hydroxo, and hydroxo-bridged polymers with loss of the acetylacetonato ligand; in methanol : water (95 : 5) the half-life is only 6 min at 22°C, and micellar preparations are used for systemic administration (*103*). This slows down the rate of hydrolysis considerably (only 20% in 4 hr). The rate of hydrolysis is also dependent on the coordinated monodentate anion:

$$OEt \ll F < Cl < Br < I$$

Like the metallocene complexes, budotitane shows little activity against P388 and L1210 leukemias, but is highly active against colon adenocarcinoma.

Although the antiarthritic complex auranofin (**14**) is highly cytotoxic to cells in culture, it is active against only one tumor model, P388 leukemia (*69*), and then only against the intraperitoneal (ip) tumor when administered ip (*105*). It is therefore not a useful anticancer agent. The high cytotoxicity of auranofin *in vitro* can be attributed to the presence of the phosphine, and its low potency *in vivo* to the high reactivity of auranofin toward thiolate ligands. In contrast, tetrahedral gold(I) diphosphine complexes such as $[Au(dppe)_2]Cl$ (**25**) (*106, 107*), where dppe is 1,2-diphenylphosphinoethane, are much less reactive toward ligand exchange and exhibit a wider spectrum of anticancer activity. The free ligands $Ph_2P(CH_2)_nPPh_2$ and dppe-bridged digold(I) complexes (**26**) exhibit a similar pattern of activity toward P388 leukemia (*108*), peaking at $n = 2$ (*109*), although the complexes are



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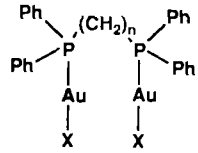

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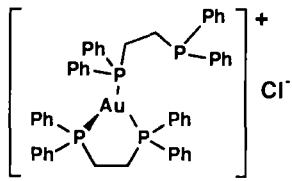
much more potent (lower doses for the same activity) than the free ligands. In blood plasma, bridged complexes are readily transformed into tetrahedral chelated bis(diphosphine) complexes (*110*). Activity is lost when the phenyl substituents are replaced by ethyls, but is retained when Au(I) is substituted by Ag(I) or Cu(I). In this case again the metal ions can be regarded as carriers for the toxic phosphine ligands, although the metal itself may play a direct role in the antitumor activity. There are facile mechanisms for ring opening that allow the ligand to act as an attacking agent (**27**). Metal diphosphine com-



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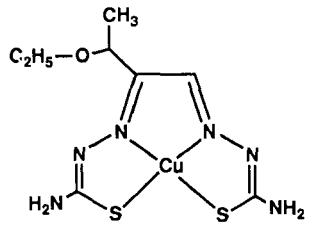
plexes appear to cause DNA strand breaks and DNA–protein cross-links, with the latter being the critical lesions at low concentrations of the complexes (*111*). Their mechanism of action is therefore different from that of cisplatin. Phosphines are strong reducing agents and do not occur naturally in biology (except for certain bacteria, which are thought to synthesize PH_3), all of which is concerned with P(V)-phosphate and phosphate ester chemistry. Metal coordination protects diphosphines from oxidation. Alkylphosphines are more readily (aut)oxidized than aryl phosphines and the nature of the products is different, being dependent on the stability of radical intermediates (*109, 112*). Besides proteins, metals in cells may be targets for diphosphines and the translocation of Cu(I) could be a key step in the activity.

No clinical trials have been carried out yet with these tetrahedral complexes because of toxic side effects on the heart and lungs, which can be attributed to attack on mitochondria (*113, 114*). $[Au(dppe)_2]Cl$ is a potent uncoupler of oxidative phosphorylation in mitochondria and

rapidly destroys electrochemical potentials across the inner mitochondria membrane, a common target site for lipophilic cations. Because it appears that the mechanisms responsible for the antitumor activity and side effects can be separated, it may be possible to redesign this series of complexes to produce one suitable for clinical trials. Our initial attempts to do this have involved increasing the aqueous solubility of gold(I) diphosphine complexes via ligand substitutions (*115*), the preparation of Ni(II) diphosphine complexes that might be more labile, and substitution of phosphorus by arsenic, which can be done isomorphously in the Au(I) complex (*116*). Again considerable attention has to be given to the form of the complexes in the biological testing media; $[Ni(dppe)Cl_2]$, for example, readily reacts with cell culture media to give rise to $[Ni(dppe)_2]^{2+}$ (*117*).

The Cu(II) bis(thiosemicarbazone) complex [Cu(KTS)] (**28**) (where KTS is 3-ethoxyl-2-oxobutyraldehyde-bisthiosemicarbazone) is a more



[CuKTS]

28

active antitumor agent than is the free ligand, and undergoes intracellular reduction to Cu(I) with release of the ligand (*118*). Again, the ligand could play a role in the translocation of Cu(I) in cells or act as a metalloenzyme inhibitor (e.g., ribonucleotide reductase). It is notable that the Cu(II) dichloride complex of the macrocyclic ligand tetrabenzo[*b,f,j,n*](*1,5,9, 13*)tetraazacyclohexadecine (TAAB) is readily reduced to the Cu(I) state, and is highly active against P388 leukemia (*119*). Similarly $[Cu(II)(2,9\text{-dimethylphenanthroline})_2]^+$, which also stabilizes Cu(I), is active in this system (*120*), but the unsubstituted phen complex is not. Cu(II) phenanthroline complexes are often used to cleave DNA *in vitro* in the presence of thiols (*121*), probably via Cu(I) and hydroxyl radical generation, but they are probably not stable enough to deliver copper to DNA *in vivo*.

An intriguing complex with antitumor activity (as well as radioprotective and antiinflammatory activity) is bis(isopropylsalicylato)Cu(II)

(*122*), which is probably a carboxylate-bridged dimer. It might be expected that it would readily be destroyed in plasma, because monodentate carboxylates complex weakly and there are many competing chelating ligands available (e.g., histidine, the N-terminal tetradentate amino acid site of albumin). However, the complex is highly lipophilic (soluble in ether), and so may partition quickly into lipophilic compartments such as lipoproteins and cell membranes. Here is a case where attention needs to be paid not only to the thermodynamics, but also to the kinetics of ligand exchange and diffusion processes.

2. Radiosensitizers

Radiation can be very effective for killing tumor cells, but some cells are hypoxic and are resistant (*16*). Dioxygen is the most effective sensitizer, acting as an electron acceptor and aiding propagation of free radical reactions that lead to DNA cleavage. Other molecules with a high electron affinity can also act as sensitizers, and could be useful in the clinic if they are sufficiently nontoxic at the doses required. Examples are the nitroimidazoles. The electron affinity of nitroimidazoles can be increased by coordination to Pt(II) (*123*), which also improves DNA targeting. Moreover, Pt(II) 5-nitroimidazole complexes have a low toxicity, in line with the absence of a hydrogen on the coordinated N in contrast to active anticancer complexes. $[PtCl_2(NH_3)(\text{nitroimidazole})]$ complexes appear to show enhanced binding to DNA compared to $[PtCl_2(\text{nitroimidazole})_2]$ complexes (*124*).

3. Antiviral Agents

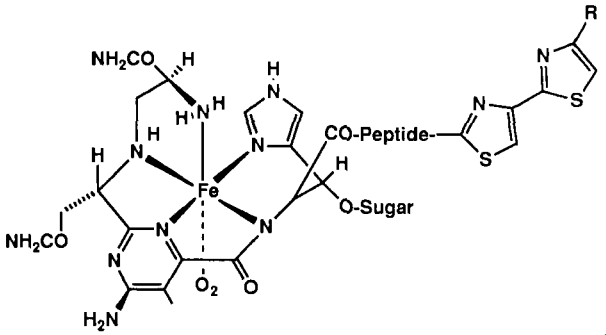
The involvement of metal ions in the replication of viruses needs to be given more attention, as does the screening of metal compounds for antiviral activity, because most current screens are optimized for organic agents. It is clear that both metal complexes and chelating agents can exhibit antiviral activity (*16*). The cryptate complex ammonium-21-tungsto-9-antimonate, $(NH_4)_{17}Na[NaW(VI)_{21}Sb(V)_9O_{86}] \cdot 14H_2O$, is active against a broad spectrum of RNA and DNA viruses, has a low toxicity, and has been on clinical trials against the human immunodeficiency virus (*125*). The complex inhibits RNA-dependent DNA polymerases (reverse transcriptases) and has a structure based on WO_6 octahedra, which form a large cavity containing an occluded sodium ion (*126*). Analogous heteropolyanions containing arsenic and phosphorus are also active. It seems likely that these cluster complexes are delivery agents for tungstate or polytungstate anions. It is notable that vanadate is a natural potent inhibitor of ATPase, and there is

much scope for the design of drugs to deliver oxyanions of this type (vanadate, molybdate, tungstate, etc.).

Cisplatin has been used as a topical agent for the treatment of herpes simplex virus, and is also active against some other viruses. Titanocene dichloride can inhibit replication of both DNA (orthopox and herpes) and RNA (rhabdovirus, paramyxovirus, and influenza A and B) viruses in the extracellular phase. In contrast, vanadocene and molybdocene dichlorides are inactive (*101*). The M—C_5H_5 bonds in vanadocene dichloride are more stable in water than are those of the Ti complex (*127*). Chelating agents such as thiosemicarbazones, diethyldithiocarbamate, and phosphonoformate are also active antiviral agents (*128*).

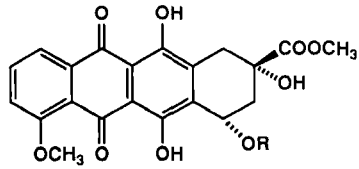
D. Metal Chelation by Organic Drugs

Metal binding can play an important role in the mechanism of action and toxic side effects of organic drugs and their metabolites, and this possibility needs to be given attention in the design process. One of the most studied examples is the anticancer drug bleomycin, which is thought to cleave DNA as an Fe(II) complex via oxygen activation (*129*) (**29**). Similarly, metals such as iron and copper may activate several antibiotics (**30**) and lead to the production of radicals (*130*).



A model for iron-bleomycin

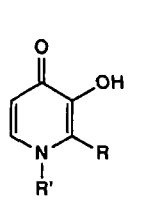
29



Adriamycin (doxorubicin)

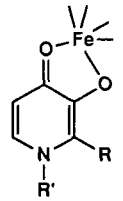
30

Ferryl intermediates formed by interaction of iron complexes with H_2O_2 may also be involved. There is a need for drugs to control the levels of circulating iron complexes following cardiac arrest. After blood flow is reestablished, lipid peroxidation reactions appear to be responsible for the damage of neural and cardiac tissue, and may well be initiated by these metals (*131*). New chelating agents such as the hydroxypyridinone derivatives (**31** and **31a**) being developed by R. Hider and colleagues (London, England) may be effective for this purpose.



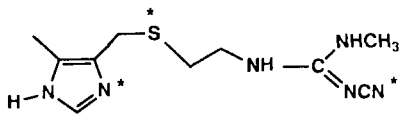
Pyridinones
e.g., R = R' = Et

31



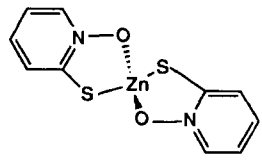
31a

The antibacterial activity of nalidixic acid appears to depend on the formation of copper or iron complexes, and the potent histamine H_2 receptor antagonist cimetidine (**32,** the antiulcer drug) forms a polymeric Cu(II) complex, being bound in the crystal by nitrogen and sulfur ligands (*132*). Metal complexes of a number of antiinflammatory agents are said to be more effective than the organic drug alone, e.g., Zn(II) ibuprofen and Cu(II) salicylates (*122, 133*). The antimicrobial agent pyrithione is marketed as a Zn(II) complex (possible structure, **33**), Omadine. The compound is active against both bacteria and fungi, and also has antidandruff properties (*134*). The ligand itself readily undergoes oxidation to the disulfide, and aqueous solutions photolyse in daylight, as do solutions of the complex, although it is not very soluble.



Cimetidine (* Cu(II) sites)

32



Zn(II) Pyrithione

33

E. Metalloenzyme Inhibitors

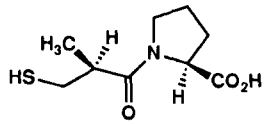
It is now emerging that a large number of key pharmaceutical targets for drugs, especially organic drugs, are metalloenzymes (Table X). In some cases the exact nature of the metal site(s) is not yet clear, and so only general features aimed at metal binding can be introduced into the inhibitor. There is space for discussion of only a few examples here.

Inhibitors of the Zn(II) hydrolase angiotensin-converting enzyme (ACE) are potentially useful for the control of hypertension and the treatment of congestive heart failure. ACE cleaves the Phe-His bond of the decapeptide angiotensin I (Asp-Arg-Val-Try-Ile-His-Pro-Phe-His-Leu) to give the octapeptide angiotensin II, a potent blood-pressure-raising substance (*135*). ACE is inhibited by chelating agents such as EDTA, *o*-phen, and dithiothreitol, and also requires chloride for activity (*136*). The orally active drug captopril (**34**) was designed to place a thiol group close to Zn(II), and now even more potent inhibitors with similarly placed carboxylates or phosphates (usually their esters as prodrugs) have been discovered (*137*).

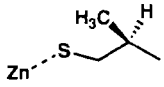
TABLE X

Some Metalloenzymes That Are Potential Targets for Organic Drugs

Enzyme	Active site	Inhibitors
Collagenase	$Zn(II)(His)_2X$? proteases	Antiarthritic agents
Stromelysin	Activated by organomercurials	Peptides (TIMP)
(Gelatinase)	Hydrolyzes collagen, α_2-macroglobulin	Au(I) [Hg(II), Cu(II)]
Angiotensin-converting enzyme	Zn(II) protease; angiotensin I → A II (vasoconstrictor)	Antihypertensives (captopril, enalapril, cilazapril)
Enkephalinase	$Zn(II)(His)_2(Glu)$? (hydrolyzes neuropeptides)	Analgesics (thiorphan, phosphoryl-Leu-Phe)
Cytochrome *P*-450	Cys—heme—Fe(V)=O; C—H → C—OH; ≡N → ≡NO; =S → =SO	Drug metabolites (benzodioxoles halothane, amphetamines, erythromycin)
Lipoxygenase (leukocytes)	$Fe(III)(His)_4$?(PQQ?)(O_2), Ca(II); 1,4-diene → 1,3-diene; hydroperoxide	Antiinflammatory, antihypersensitivity agents (acetohydroxamates)
Prolyl 4-hydoxylase	Fe(III) (O_2)(2-oxoglutarate); Pro → 4-OH—Pro (collagen, C_{1q})	Fibroproliferative disorders (carboxypyridines)
Ribonucleotide reductase	Fe(III)—Glu—Fe(III) (Tyr radical); ribose → deoxyribose	Anticancer agents (hydroxyureas)
Dopamine-β-hydroxylase	Cu(II) (O_2); dopamine → noradrenaline	Neurodisorders (benzylimidazole-2-thiols)



34 Captopril

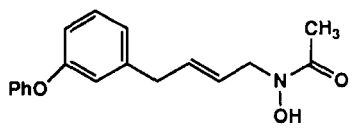


34a

Similarly, no crystal structure is available yet for the important class of Zn(II) enzymes that hydrolyze collagen. Collagenase has some very unusual properties, being activated by organomercurials, and Mallya and Van Wart have reported a remarkable inhibition of human neutrophil collagenase by the antiarthritic drugs aurothiomalate and aurothioglucose at nanomolar concentrations (*138*). The activity of such enzymes *in vivo* may be finely controlled by local concentrations of Zn(II), Cu(I), and inhibitors such as Au(I).

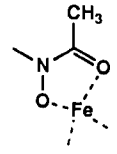
Enkephalinase is found in the brain as well as in other tissues and is localized in synaptic membranes enriched in opiate receptors (*139*). It is also a Zn(II) protease, with some sequence homology to carboxypeptidase, and hydrolyzes the Gly^3-Phe^4 bond of enkephalins (Tyr-Gly-Gly-Phe-Met and Tyr-Gly-Gly-Phe-Leu) into inactive fragments. Inhibitors are potentially useful as analgesic drugs.

Lipoxygenase has been isolated from leukocytes, mast cells, and leukemia cells and catalyzes the peroxidation by dioxygen of fatty acids containing the 1,4-diene unit. It is involved in the biosynthesis of leukotrienes, a group of arachidonic-acid-derived metabolites that have important roles in inflammation and immediate hypersensitivity. For example, leukotrienes LTC_4 and LTD_4 are powerful bronchoconstrictors and increase vascopermeability, and elevated levels have been found in patients with asthma, rheumatoid arthritis, and psoriasis. The nature of the active site of lipoxygenase is unclear at present, although iron is required together with O_2, Ca^{2+}, ATP, and perhaps pyrroloquinoline-quinone (PQQ). Some success has been reported in designing orally active inhibitors (**35** and **35a**) with features similar to



Orally active inhibitor of lipoxygenase

35



35a

either substrate or product, and an additional hydroxamic acid function that binds to the iron (*140*).

Cytochrome *P*-450 is involved in the biosynthesis and degradation of endogenous compounds such as steroids, fatty acids, prostaglandins, and leukotrienes, and the oxidative metabolism of drugs and xenobiotics. It is a monooxygenase, and the site of dioxygen activation is a protoporphyrin IX heme group linked to the protein via an Fe–cysteine thiolate sulfur bond. A key intermediate in the mechanism is an Fe(V)=O[Fe(IV)—O·] species (see Ref. *141*). Stable iron-metabolite complexes are formed during the metabolism of many drugs; these include amphetamines, propoxyphene, erythromycin, and troleandomycin, and some can be detected in the livers of humans treated with antibiotics. They can be N bound, as in the latter case, or C bound, as for the anesthetic halothane. There are many isozymes of cytochrome *P*-450 and, in future, these will be produced by recombinant DNA technology so that the products of drug metabolism in the body can be predicted with more certainty and new inhibitors can be designed.

V. Conclusion

Inorganic chemistry has much to contribute to modern pharmacology. In particular, it introduces the potential for the discovery for truly novel drugs. In this article I have concentrated mainly on metals, and have adopted a three-part classification of therapeutic and diagnostic agents. The activity of a metal complex may require that at least some of the ligands remain bound to the metal when it reaches the target site; alternatively, the ligands may just provide a vehicle for delivery and not be critical for activity. At the other extreme, the ligands themselves may be the active species. The classification that I have introduced is not intended to be a rigid one; some agents can be placed in more than one category and some others are placed with ignorance of their mechanism of action. Even when a metal delivers an active ligand, it may itself be translocated to an unusual site, and vice versa. These arguments need to be extended to nonmetals, for which ligand exchange and redox processes are equally important in their pharmacology. Indeed, interest in nitrogen is increasing daily, with nitric oxide not only playing a role as a muscle relaxant, but now as a neurotransmitter, being released upon nervous stimulation of gastrointestinal tissue (*142*).

At least 25 elements are essential for life, and, in the future, pharmacology will be concerned with the uptake, metabolism and excretion of all of them. Also, compounds of most of the other elements of the Periodic Table can be designed to have a specificity of biological activity. Often it will be possible to control the metabolism of essential elements with organic drugs, because there is frequently an intimate synergism between the action of inorganic elements and organic compounds in the body. Metals are known to control the activity of enzymes that are key pharmaceutical targets; indeed, metals control some of the most fundamental biochemical processes, including the structure and replication of DNA and RNA. Therapy needs to become more sophisticated, e.g., by more frequent use of combinations of drugs (organic plus inorganic) and by monitoring the effects of drugs on the distribution of the elements.

Some inorganic compounds are already successful drugs, others need a much greater attention to be devoted to their chemistry and biochemistries, examples of the latter being antimony antileishmanial drugs and bismuth antiulcer drugs. There is an urgent need for new methods and new strategies to be devised for the testing of inorganic compounds. Existing tests for organic compounds do not provide an adequate sieve for active inorganic compounds, and antiviral testing is a particular case here. New techniques and methods are also required for investigating the molecular pharmacology of inorganic compounds, ranging from analytical determinations of the elements (especially multielement analyses) to studies of coordination chemistry in intact biological materials. A greater emphasis needs to be given to the rates of processes involving metal ions, and not just the positions of equilibria (stability and thermodynamics). In biology the fastest processes are often the most important.

Currently, for every successful drug, 10,000 candidates are tested, and in the anticancer field, out of every 10,000 compounds tested only 6 to 8 reach clinical trials (*143*). It is therefore evident that strategies for drug design need to improve. One immediate way to do this is to give inorganic chemistry a much greater weighting in the design process. In the United States alone in 1989, 22 new chemicals were approved for pharmaceutical use at a cost of $14,000 million (*144*), nearly all of which was spent on organic chemistry. However, "A huge world of inorganic drugs—which has been ill-explored since organic chemists dominate biochemical practice—is slowly opening" (*1*), and this will provide the stimulation for much new and exciting inorganic chemistry in the future.

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