

Immediate (type I) allergic responses to platinum compounds

M. J. CLEARE,* E. G. HUGHES,* B. JACOBY† and J. PEPYS†

* Johnson Matthey & Co. Ltd, 78 Hatton Garden, London EC1, and † Department of Clinical Immunology, Cardiothoracic Institute, Brompton, London SW3

Summary

A series of platinum complexes has been used for allergy tests in a number of platinum refinery workers who are known to be sensitive to hexachloroplatinate ($[\text{PtCl}_6]^{2-}$) salts. The results show that the allergy-eliciting compounds are confined to a very small group of ionic complexes containing reactive halogen ligands. Neutral complexes and those containing more strongly bound ligands are totally inactive. These variations in activity are interpreted in terms of established kinetic and thermodynamic parameters.

Introduction

There are several reports in the literature that complex salts of platinum are potent sensitizers and highly allergenic to man (Hunter, Milton & Perry, 1945; Roberts, 1951; Parrot *et al.*, 1969). The studies have been confined until recently to one type of complex only, namely halide complexes hexachloroplatinate IV $[\text{PtCl}_6]^{2-}$, and tetrachloroplatinate II $[\text{PtCl}_4]^{2-}$. An impression has, however, been created that all platinum compounds have similar properties. Halide complexes of platinum are encountered mainly by chemists and workers engaged in the refining of platinum and their allergenicity is shown by sensitization, presumably by the inhalation route of the exposed subjects and particularly of atopic subjects. (Webster; Hughes; personal communications.)

The minute doses capable of eliciting reactions in sensitized subjects are further evidence of their allergenic potency (Pepys, Pickering & Hughes, 1972). Sensitized subjects present with rhinitis, conjunctivitis and asthma, and to a lesser extent with urticaria and contact dermatitis. The allergenic properties of platinum compounds have become of more general clinical interest, following on the introduction of anti-tumour platinum co-ordination compounds by Rosenberg *et al.* (1969) and by the reports by Khan *et al.* (1975) and von Hoff *et al.* (1976) of anaphylaxis in a small number of patients undergoing intravenous treatment with the platinum co-ordination compound *cis*-dichlorodiammineplatinum (II) as *cis*- $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2]$.

The allergenicity of certain platinum salts provides a unique opportunity for the

Correspondence: Professor J. Pepys, Department of Clinical Immunology, Cardiothoracic Institute, Brompton, London SW3.

investigation of a group of well-defined small molecular inorganic agents. In general, small molecular allergenic substances act as haptens, becoming fully antigenic on combination with large molecular carrier substances, mainly protein, for the stimulation of antibody production and elicitation of reactions to testing.

Extensive attempts in our laboratories to demonstrate relevant antibodies against platinum halide complexes, known to be sensitizers, and their conjugates have had only limited success.

Little is known in general about the actual hapten/carrier linkages in terms of allergenicity for the elicitation of reactions. If a reaction of the platinum salt with a carrier or carriers is involved, it seemed likely that the strength of the platinum ligand bond and the reactivity of the complex towards protein or other carrier molecules would largely determine its ability to elicit allergic reactions.

Recourse has therefore been made to skin prick tests under carefully controlled conditions for eliciting Type I reactions to a range of platinum complexes in Platinum Refinery workers, known to be sensitive to $[\text{PtCl}_6]^{2-}$ and $[\text{PtCl}_4]^{2-}$ salts. The aim of these tests was to correlate the structure of the complex with its ability to elicit a reaction and to establish the degree to which the various types of platinum complexes exhibit this property.

Materials and methods

Skin prick tests

Prick tests were made by introducing the tip of separate small (No. 26 disposable) hypodermic needles at a low angle into the epidermis of the forearm with a gentle lifting motion through drops of the test solutions on the skin. Reactions were read after 10 min. There is no wealing at control tests except in dermatographic subjects, so that even small positive reactions could be readily assessed. This highly precise test is used chiefly for the demonstration of Type I allergy and introduces according to Squire (1952) a volume of 3×10^{-6} ml into the epidermis. The prick test is at present used routinely for pre-employment assessment of atopic status in terms of extracts of relevant common allergens and for serial observation for the development of sensitivity, for example, to the platinum salts.

Selection of skin test method

The method of skin testing with these potentially allergenic materials has to be selected with care to avoid unduly severe reactions and non-specific reactions. Intracutaneous tests may be hazardous and there are reports of severe systemic reactions even to scratch tests (Freedman & Krupey, 1968; Levene & Calnan, 1971). Some of the platinum salts are also histamine liberators (Parrot *et al.*, 1969). The skin prick test (Squire, 1952) is not only safer for this purpose (Pepys *et al.*, 1972), but it also provides a greater margin between test concentrations for eliciting specific allergic reactions and those eliciting non-specific reactions for example to histamine. The threshold concentration in the intracutaneous test for eliciting wealing reactions to histamine or histamine liberators is from 10^3 to 10^4 lower than for the prick test (Pepys, personal communication). Scratch tests were used by Roberts (1951) who found that all subjects reacted to scratch tests with concentrations of 10^{-1} , 10^{-2} g/ml of sodium chloroplatinate, whereas affected subjects reacted to 10^{-3} to 10^{-8} concentrations. Using the prick test

we found that a 10^{-3} g/ml concentration gave negative reactions in all non-exposed, non-sensitive subjects.

Test preparations

Solutions of the complexes were prepared in glycerol/water (1:1) or Coca's fluid plus 50% glycerol. A stock solution of 10^{-3} g/ml was made, with ten-fold dilutions down to 10^{-9} g/ml. Four solutions of different salts were tested at a time at marked sites across the forearm, starting with the 10^{-9} g/ml solution. The mean diameter of positive reactions was measured in mm. If there was no reaction to the particular preparation within 10 min, the next concentration was tested and so on up to the final concentration of 10^{-3} g/ml. In each series of tests $(\text{NH}_4)_2 \text{PtCl}_6$ was used as a positive control.

Preparation of complexes

The compounds were all prepared by established methods cited in the literature as indicated and had satisfactory elemental analyses. As the tests were carried out on refinery workers, some of whom are known to be very sensitive to $[\text{PtCl}_6]^{2-}$ or $[\text{PtCl}_4]^{2-}$ salts, there exists the problem that slight contamination with these ions (not detectable by elemental analysis) could lead to a positive response albeit at a much higher concentration (for example, a person sensitive to $[\text{PtCl}_4]^{2-}$ at 10^{-6} g/ml would show a positive response to a different complex at 10^{-3} g/ml if it contained 0.1% of $[\text{PtCl}_4]^{2-}$). Every effort was made to minimize such contamination and the large number of negative responses indicate that this was successful.

(A) *Halide complexes.* $(\text{NH}_4)_2 [\text{PtCl}_6]$ and $(\text{NH}_4)_2 [\text{PtCl}_4]$ were commercial samples as sold by Johnson Matthey Chemicals Ltd.

(i) *Ammonium hexabromoplatinate (IV)*, $(\text{NH}_4)_2 [\text{PtBr}_6]$. A commercial sample was evaporated ($2 \times$) from dilute hydrobromic acid (Analar), and then recrystallized from the same solvent. Chloride contamination was $<0.01\%$.

(B) *Nitro complexes.*

(ii) *Dicaesium trichloronitroplatinate (II)*, $\text{Cs}_2[\text{Pt}(\text{NO}_2)\text{Cl}_3]$. This was prepared by the addition of the stoichiometric amount of potassium nitrite, KNO_2 , to a hot solution of $\text{K}_2[\text{PtCl}_4]$ in a minimum amount of water (Chernyaev, 1928; Cleare, 1970). Caesium chloride CsCl , was added to the filtrate to give a yellow-orange precipitate which was recrystallized from hot water. This compound is very soluble and there is a good chance of some $[\text{PtCl}_4]^{2-}$ contamination. Thus this complex was prepared and tested on two separate occasions, with sample (a) being recrystallized twice and samples (b) and (c) three times from water. Analyses indicated slight contamination with $\text{Cs}_2[\text{Pt}(\text{NO}_2)_2\text{Cl}_2]$ but not $[\text{PtCl}_4]^{2-}$.

(iii) *Dicaesium dichlorodinitroplatinate (II)*, $\text{Cs}_2[\text{Pt}(\text{NO}_2)_2\text{Cl}_2]$. Prepared as for (ii) from stoichiometric amounts of KNO_2 and $\text{K}_2[\text{PtCl}_4]$ followed by the addition of CsCl (Cleare, 1970). The yellow complex was recrystallized from water. It is less soluble than (ii) and the chance of significant $[\text{PtCl}_4]^{2-}$ contamination is much less as $\text{Cs}_2[\text{PtCl}_4]$ is fairly soluble.

(iv) *Dicaesium chlorotrinitroplatinate (II)*, $\text{Cs}_2[\text{Pt}(\text{NO}_2)_3\text{Cl}]$. Prepared as in (ii) from stoichiometric amounts of KNO_2 and $\text{K}_2[\text{PtCl}_4]$ followed by the addition of CsCl (Cleare, 1970). The pale yellow complex was recrystallized from water. The aqueous solubility is much lower than for (ii) or (iii) and the possibility of significant

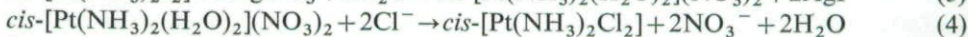
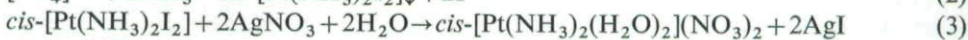
$[\text{PtCl}_4]^{2-}$ contamination is very small. The analyses indicated the presence of a small amount of $\text{Cs}_2[\text{Pt}(\text{NO}_2)_4]$.

(v) *Dipotassium tetranitroplatinate (II)*, $\text{K}_2[\text{Pt}(\text{NO}_2)_4]$. Aqueous solutions of KNO_2 (excess) and $\text{K}_2[\text{PtCl}_4]$ were mixed and heated until the mixture was virtually colourless (Matthieu & Cornevin, 1939). White needles of the product formed on cooling, and these were recrystallized from hot water. Again on solubility grounds, contamination is likely to be extremely low (confirmed by the negative results).

(C) *Amine complexes.*

(vi) *Tetrammineplatinum (II) trichloroammineplatinate (II)*, $[\text{Pt}(\text{NH}_3)_4][\text{PtCl}_3(\text{NH}_3)]$. This was prepared by the method of Elleman (1958). *Cis*- $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2]$ was refluxed with concentrated HCl in the presence of some reduced platinum catalyst. The mixture was cooled and unchanged *cis*- $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2]$ removed by filtration. The filtrate was reduced in bulk and $[\text{Pt}(\text{NH}_3)_4]\text{Cl}_2$ was added to give green yellow crystals, consisting of the product plus some Magnus salt, $[\text{Pt}(\text{NH}_3)_4][\text{PtCl}_4]$, which is green in colour. The crude product was recrystallized ($2\times$) from hot water containing a little $[\text{Pt}(\text{NH}_3)_4]\text{Cl}_2$. The Magnus salt has extremely low solubility and remains behind after the first recrystallization. Because of this the possibility of significant $[\text{PtCl}_4]^{2-}$ contamination is slight.

(vii) *Cis-dichlorodiammineplatinum (II)*, *cis*- $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2]$. This was prepared via the corresponding iodo complex using the following route (Dhara, 1970; Cleare & Hoeschele, 1973).

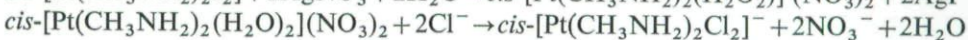
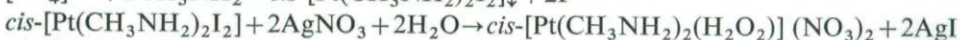
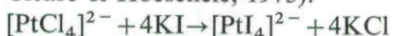


Potassium tetraiodoplatinate (II), $\text{K}_2[\text{PtI}_4]$ was prepared in solution by the addition of a stoichiometric quantity of potassium iodide, KI , to an aqueous solution of $\text{K}_2[\text{PtCl}_4]$. After a few minutes aqueous ammonia was added to afford a yellow precipitate of the insoluble iodo complex, *cis*- $[\text{Pt}(\text{NH}_3)_2\text{I}_2]$ in virtually quantitative yield. This was converted to the aquo species by reaction with a solution of silver nitrate, AgNO_3 , according to equation (3). The filtered solution was converted to the chloro complex, *cis*- $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2]$ by the addition of concentrated HCl . The product was carefully purified by dissolution in dimethylformamide (DMF) followed by reprecipitation with dilute HCl . The final precipitate was thoroughly washed with 0.1 N HCl and water.

The iodo complex is very insoluble and this plus the purification stage should ensure that contamination with $[\text{PtCl}_4]^{2-}$ is negligible.

(viii) *Trans-Dichlorodiammineplatinum (II)*, *trans*- $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2]$. This was prepared by the action of boiling concentrated HCl on $[\text{Pt}(\text{NH}_3)_4]\text{Cl}_2$ for several hours (Kauffman & Cowan, 1963). The initial product was recrystallized by dissolving in dimethylformamide (DMF) and adding excess dilute HCl . The pure product was thoroughly washed with water to remove all traces of DMF. Contamination with $[\text{PtCl}_4]^{2-}$ would again be negligible.

(ix) *Cis-dichlorobis(methylamine)platinum (II)*, *cis*- $[\text{Pt}(\text{MeNH}_2)_2\text{Cl}_2]$. This was prepared using the following route analogous to that used for (vii) (Dhara, 1970; Cleare & Hoeschele, 1973).



The method was identical to that described for (vii) except that methylamine (40% aqueous solution) was used instead of ammonia. Again contamination with $[\text{PtCl}_4]^{2-}$ should be negligible.

(x) *Trans-dichlorobis(methylamine)platinum (II)*, $\text{trans-}[\text{Pt}(\text{CH}_3\text{NH}_2)_2\text{Cl}_2]$. As in (viii) the method involved the preparation of a solution containing the fully substituted tetramine, in this case $[\text{Pt}(\text{CH}_3\text{NH}_2)_4]\text{Cl}_2$, by the action of excess methylamine on $\text{K}_2[\text{PtCl}_4]$ in the presence of methylamine hydrochloride (Cleare & Hoeschele, 1973). The action of boiling concentrated HCl on this tetramine solution yielded the product as pale yellow crystals which were recrystallized exactly as in (viii). Again, any contamination with $[\text{PtCl}_4]^{2-}$ would be negligible.

(xi) *Cis-dichlorobis(ethanolamine)platinum (II)*, $\text{cis-}[\text{Pt}(\text{OHC}_2\text{H}_4\text{NH}_2)_2\text{Cl}_2]$. Again prepared by the iodo route described for (viii) but using ethanolamine (2-aminoethanol) instead of ammonia (Cleare & Hoeschele, 1973). The iodo complex $\text{cis-}[\text{Pt}(\text{OHC}_2\text{H}_4\text{NH}_2)_2\text{I}_2]$ is more soluble than in (viii) but still forms in 90% yield. The chloro complex is soluble compared to (viii) or (x) and is formed as pale yellow crystals in a 40% yield. It was recrystallized from 3 N HCl. The possibility of contamination is higher than for (viii) or (x) but should still be very low.

(xii) *Trans-dichlorobis(ethanolamine) platinum (II)*, $\text{cis-}[\text{Pt}(\text{OHC}_2\text{H}_4\text{NH}_2)_2\text{Cl}_2]$. As for the other *trans* compounds, the tetramine $[\text{Pt}(\text{OHC}_2\text{H}_4\text{NH}_2)_4]\text{Cl}_2$ was first prepared by the action of excess amine, $\text{K}_2[\text{PtCl}_4]$ in hot aqueous solution (Cleare, 1974). The colourless solution which formed was boiled with concentrated HCl to yield the product which was recrystallized from 2 N HCl. This complex is considerably less soluble than the *cis* isomer and contamination should be extremely low.

(xiii) *Iodo(diethylenetriamine)platinum (II) iodide*, $[\text{Pt}(\text{dien})\text{I}]$. This complex was prepared from $\text{PtI}_2 \cdot \text{H}_2\text{O}$ which is precipitated when potassium iodide is added to a hot solution of $\text{K}_2[\text{PtCl}_4]$ (Watt & Cude, 1968). $\text{PtI}_2 \cdot \text{H}_2\text{O}$ and diethylenetriamine were mixed together with a little water and evaporated to dryness on a steam bath. The residue was extracted with hot water and evaporated to give yellow crystals of the product. These were recrystallized twice from water plus activated charcoal.

(xiv) *Bromo(diethylenetriamine)platinum (II) bromide*, $[\text{Pt}(\text{dien})\text{Br}]\text{Br}$ (Watt & Cude, 1968). $[\text{Pt}(\text{dien})\text{I}]\text{I}$ (xiii) was converted to the aquo species by reaction with aqueous silver nitrate.



The bromo complex was formed by the addition of concentrated HBr. The pale yellow product was recrystallized from potassium bromide, KBr, solution after charcoal treatment.

(xv) *Chloro(diethylenetriamine)platinum (II) chloride*, $[\text{Pt}(\text{dien})\text{Cl}]\text{Cl}$ (Watt & Cude, 1968). Prepared as for the bromo complex via the aquo species. The chloro complex was precipitated using concentrated HCl. The very soluble white product was recrystallized twice from dilute HCl after a charcoal treatment. All of the dien salts (xiii, xiv, xv) are very soluble and every effort was made to minimize $[\text{PtCl}_4]^{2-}$ contamination.

(xvi) *Tetrammineplatinum (II) chloride*, $[\text{Pt}(\text{NH}_3)_4]\text{Cl}_2$. Prepared as by Keller (1946) by the action of excess ammonia on $\text{K}_2[\text{PtCl}_4]$. Recrystallized from very dilute ammonia solution. Contamination with $[\text{PtCl}_4]^{2-}$ should be low as this is removed as Magnus green, $[\text{Pt}(\text{NH}_3)_4][\text{PtCl}_4]$ which is very insoluble.

(D) Others.

(xvii) *Tetrakis(thiourea)platinum (II) dichloride*, $[\text{Pt}(\text{NH}_2)_2\text{CS}_4]\text{Cl}_2$. This pre-

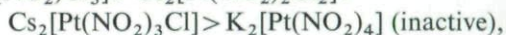
paration forms part of the basis of the classical Kurnakov test for distinguishing between *cis* and *trans* isomers of platinum diamines (Kauffman & Cowan, 1963; Kurnakov, 1894). *Cis*-[Pt(NH₃)₂Cl₂] reacts with excess aqueous thiourea on boiling to give a deep yellow solution which deposits [Pt(tu)₄]Cl₂ (tu = thiourea) on cooling. The *trans* isomer only proceeds to *trans*-[Pt(tu)₂Cl₂] (white crystals). In the *cis* case the strong *trans* effect of the S-bonded thiourea ligands labilizes the amine groups and allows them to be replaced. The yellow crystals of [Pt(tu)₄]Cl₂ were recrystallized from water. Contamination with [PtCl₄]²⁻ should be very low (as is confirmed by the negative tests).

(xviii) *Caesium dichloroglycinatoplatinate (II)*, Cs[Pt(glycine)Cl₂]. The method of Erickson *et al.* (1968) was employed. An aqueous solution of an equimolar mixture of K₂[PtCl₄] and glycine was heated on a steam bath and the falling pH maintained at 4 by the addition of 1 equivalent of KOH per mole of glycine. The solution was evaporated to dryness and extracted with hot 95% ethanol and allowed to crystallize to give yellow crystals. These were dissolved in a minimum volume of water and excess CsCl added to give pale yellow crystals on cooling. These were recrystallized from water. Contamination with [PtCl₄]²⁻ is possible but should be small.

Results

These are presented in Table 1. The process operators tested are designated A to R and the concentration at which they showed a positive reaction is indicated for each complex. The figures in parenthesis refer to the size in millimetres of the weal produced. A small positive reaction (indicated by a flare and no weal) is denoted by (+) and a less definite reaction (a smaller flare) by $\pm$. No reaction at the highest dose tested is indicated by (-). Operators Q and R act as negative controls as they do not react to the positive control, (NH₄)₂[PtCl₆], and are also not sensitive to the other compounds. Positive reactions were elicited to the lowest concentration tested, i.e. 10⁻⁹ g/ml. The volume introduced by the prick test is 3 $\times$ 10⁻⁶ ml so that the absolute skin test dose in these tests was less than 10⁻¹⁵ g, calculated at about 100,000 molecules.

It is immediately noticeable that the allergic response is maintained whenever at least one chloro ligand is presented in a charged complex. Even allowing for the inaccuracies inherent in this type of measurement, it seems that the number of chloro groups help to determine the degree of allergenicity. Thus the following sequence;



holds in nearly every case. The amine complexes show the same general pattern although with one or two exceptions which may not be significant. An important difference is that the dichloro complexes (both *cis* and *trans* [Pt(A)₂Cl₂] where A = NH₃, MeNH₂) are neutral and show no allergic activity. The only exception to this was *cis*-[Pt(OHC₂H₄NH₂)Cl₂] which elicited a response in some cases at the highest concentration. This compound is very soluble in comparison to the other neutral species and may suffer from very slight contamination, 0.001% of [PtCl₄]²⁻ would be sufficient.

A charged species containing two *cis* chloro ligands [Pt(gly)Cl₂]⁻ was slightly active. Amine species with one chloro group were either slightly active or inactive, irrespective of charge (i.e. [Pt(dien)Cl]⁻, [Pt(NH₃)Cl₃]⁻, [Pt(NH₃)₃Cl]⁺—although there may be a contamination problem with the latter).

Table 1. Prick test results

Platinum complex	Test series	Test concentration with mean diameter (mm) weal size in brackets															
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P Q R
(NH ₄) ₂ [PtCl ₆]	(a)	10 ⁻⁴ (3)	10 ⁻⁵ (3)	10 ⁻⁶ (3)	10 ⁻⁷ (+)	10 ⁻⁵ (2)	10 ⁻⁵ (2)	10 ⁻⁵ (2)	10 ⁻⁶ (2)	10 ⁻⁶ (±)	10 ⁻⁵ (1)	10 ⁻⁶ (2)	10 ⁻⁵ (1)	10 ⁻⁷ (1)	10 ⁻⁵ (2)	10 ⁻⁶ (3)	0 0
	(b)	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷ (2)	10 ⁻⁸ (2)	10 ⁻⁴	10 ⁻⁵	10 ⁻⁷ (2)	10 ⁻⁶ (3)	10 ⁻⁹ (2)	10 ⁻⁶ (3)	10 ⁻⁷	10 ⁻⁴	10 ⁻⁷ (3)	10 ⁻⁷ (4)		0
	(c)			10 ⁻⁵	10 ⁻⁸ (3)	10 ⁻⁶ (2)		10 ⁻⁵	10 ⁻⁷	10 ⁻⁷	10 ⁻⁶ (3)	10 ⁻⁷					
	(d)			10 ⁻⁷	10 ⁻⁹ (4)				10 ⁻⁹ (2)	10 ⁻⁹ (2)	10 ⁻⁶ (3)	10 ⁻⁷	10 ⁻⁴				
(NH ₄) ₂ PtCl ₄ (NH ₄) ₂ [PtBr ₆] Cs ₂ [Pt(NO ₂) ₂ Cl ₂]	(a)	10 ⁻⁶	10 ⁻⁶	10 ⁻⁵	10 ⁻⁸	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁷	10 ⁻⁷	10 ⁻⁷					
	(b)	10 ⁻⁴ (+)	10 ⁻⁵ (+)	10 ⁻⁵ (3)	10 ⁻⁶ (1)	10 ⁻³ (4)		10 ⁻⁴ (2)	10 ⁻⁴ (±)	10 ⁻⁴ (2)	10 ⁻⁴ (3)	10 ⁻⁴ (±)	10 ⁻³ (2)	10 ⁻⁵ (3)	10 ⁻⁴ (1)	10 ⁻⁴ (4)	0 0
	(c)			10 ⁻⁵ (2)	10 ⁻⁶ (1)			10 ⁻⁵ (2)	10 ⁻⁵ (1)	10 ⁻⁷ (3)	10 ⁻³ (3)	10 ⁻⁶ (3)					0
Cs ₂ Pt(NO ₂) ₂ Cl ₂ Cs ₂ [Pt(NO ₂) ₂ Cl]	(a)	10 ⁻³ (±)	10 ⁻⁴ (2)	10 ⁻⁴ (2)	10 ⁻² (2)					10 ⁻⁴ (2)	10 ⁻³ (2)	10 ⁻³ (3)	10 ⁻³ (±)				0
	(b)	10 ⁻³ (±)	10 ⁻³ (2)	10 ⁻³ (1)	10 ⁻³ (3)	0				10 ⁻⁴ (2)	10 ⁻³ (2)	10 ⁻³ (3)	10 ⁻³ (±)	10 ⁻³ (3)	10 ⁻³ (3)	10 ⁻³ (3)	0
	(c)			10 ⁻⁴ (3)	10 ⁻⁶ (2)					10 ⁻⁴ (1)	10 ⁻⁵ (3)	10 ⁻⁴ (2)	10 ⁻⁴ (2)	10 ⁻⁴ (2)	10 ⁻³ (2)		0
K ₂ Pt(NO ₂) ₄ cis-[Pt(NH ₃) ₂ Cl ₂] trans-[Pt(NH ₃) ₂ Cl ₂] cis-[Pt(CH ₃ NH ₂) ₂ Cl ₂] trans-[Pt(CH ₃ NH ₂) ₂ Cl ₂] [Pt(OH Et NH ₂) ₂ Cl ₂]	i. cis																
	ii. trans																
	cis-[Pt(NH ₃) ₂ (H ₂ O) ₂](NO ₂) ₂																
[Pt(dien)X]X (i) X = Cl (ii) X = Br (iii) X = I [Pt(NH ₃) ₂ Cl ₂] [Pt(tu) ₂ Cl ₂] Cs[Pt(gly)Cl ₂]																	

(a), (b), (c), (d) for (NH₄)₂[PtCl₆] represent the results of different control tests in the order in which they were carried out.

F, Cs₂[Pt(NO₂)₂Cl₂] and Cs₂[Pt(NO₂)₂Cl], (a), (b), (c) represent different samples, prepared and purified separately.

* On this occasion the subject had taken an antihistamine preparation prior to the test, tu = thiourea, (NH₂)₂CS; OH Et NH₂ = ethanolamine; OH C₂ H₄ NH₂; gly = glycine anion, H₂C(NH₂)COO⁻.

Weal sizes omitted where not available.

A fully substituted ammine, $[\text{Pt}(\text{NH}_3)_4]\text{Cl}_2$, was totally inactive as was $[\text{Pt}(\text{tu})_4]\text{Cl}_2$, another complex in which all the co-ordinated chlorides have been replaced.

Changing from chloro to bromo ligands maintains the response but at an apparently reduced level. Thus $(\text{NH}_4)_2[\text{PtCl}_6] > (\text{NH}_4)_2[\text{PtBr}_6]$ was observed in all cases. $[\text{Pt}(\text{dien})\text{Br}]\text{Br}$ and $[\text{Pt}(\text{dien})\text{I}]\text{I}$ were both inactive as was the corresponding chloro complex.

Discussion

The high degree of allergic sensitivity to the platinum salts ($[\text{PtCl}_6]^{2-}$ and $[\text{PtCl}_4]^{2-}$) and their possible histamine liberating effect makes it essential that the methods of testing be as safe and as free as possible from the production of non-specific reactions, as with the prick test. It also means that very low levels of contamination of a test product, even of the order of 10^{-6} with for example, $(\text{NH}_4)_2[\text{PtCl}_6]$ or $(\text{NH}_4)_2[\text{PtCl}_4]$ may suffice to give a positive reaction at a test concentration of 10^{-3} g/ml in a subject reacting to the 'contaminant' at 10^{-9} g/ml. Indeed, an impurity of this order would have to be excluded as a possible cause of the reactions reported by Khan *et al.* (1975) to *cis*- $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2]$.

In our experience the refinery workers sensitized probably by inhalation of $[\text{PtCl}_6]^{2-}$ and $[\text{PtCl}_4]^{2-}$ salts differ in their cross-reactivity from the patient reported by Khan *et al.* (1975). Their patient had an anaphylactic reaction to the seventh intravenous injection of *cis*- $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2]$ after 9 months' treatment, and gave positive skin test reactions to *cis*- $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2]$ and *cis*- $[\text{Pt}(\text{NH}_3)_2\text{I}_2]$ as well as $\text{K}_2[\text{PtCl}_6]$ and $\text{K}_2[\text{PtCl}_4]$. Positive reactions were also elicited by the diaquo derivative *cis*- $[\text{Pt}(\text{NH}_3)_2(\text{H}_2\text{O})_2]^{2+}$, a reactive species without halide ligands. The platinum refinery workers did not react to either *cis*- $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2]$ or the diaquo species *cis*- $[\text{Pt}(\text{NH}_3)_2(\text{H}_2\text{O})_2]^{2+}$.

Typical Type I skin, nasal and bronchial reactions can be elicited with solutions of the platinum halide complex salts (Pepys *et al.*, 1972) suggesting that mast cell sensitizing antibody, presumably IgE, is present. This is supported by the report of Freedman & Krupey (1968) on passive transfer of sensitivity with the serum of a highly sensitive platinum chemist. The production of serum-sickness-like reactions, similar to those of Type III allergy in a highly sensitive subject undergoing hyposensitization treatment with platinum chloroplatinate, suggests that precipitating antibodies may also be present (Levene & Calnan, 1971). In both of these cases severe general reactions were elicited by skin testing emphasizing the need for great care with these highly allergenic substances. In neither case, however, were antibodies demonstrated by serological tests.

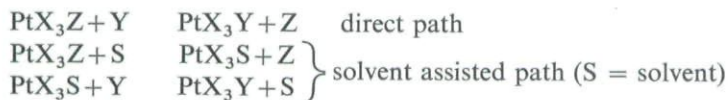
Our attempts to demonstrate specific IgE antibodies in RAST tests, and PCA tests in the monkey have all given negative results. The platinum halide complexes ($[\text{PtCl}_4]^{2-}$ and $[\text{PtCl}_6]^{2-}$) combine with proteins so that non-specific reactions with immunoglobulins may occur if they are used in too high concentrations. On the other hand, with too low concentrations, the number of molecules of platinum salt available for antibody interaction may be too low and spatially unsuitable. For example, prick tests in a highly sensitive subject, reacting to 10^{-9} g/ml concentrations of $(\text{NH}_4)_2[\text{PtCl}_4]$ and $(\text{NH}_4)_2[\text{PtCl}_6]$, with equimolar conjugates of these salts with human serum albumin, transferrin and ovalbumin, and with a conjugate of human gammaglobulin of 3 mols protein/platinum salt were negative. The platinum content of these conjugates ranged from 6 to 27 p.p.m. This raises questions about the nature

of the carrier protein when the salts themselves are used for testing, and on whether the spatial arrangements of the platinum ions were such as to enable bridging of IgE antibody molecules on the surface of the skin mast cells. It has been found by Ishizaka & Ishizaka (1971) that 7S molecules of about 30,000 molecular size are necessary for this bridging, which triggers off the release of histamine from the cells. It is conceivable that the platinum conjugates did not fulfil this requirement.

RAST tests in our own laboratory have been used with a wide range of conjugates for the presence of specific IgE antibody to platinum conjugates. In spite of the high degree of Type I skin test reactivity to the platinum salts, it has not been possible to demonstrate IgE antibodies. The agents used so far for linking to paper discs for the RAST test, include conjugates of $(\text{NH}_4)_2\text{PtCl}_4$ and $(\text{NH}_4)_2\text{PtCl}_6$ with human, bovine and rabbit serum albumin, ovalbumin, gelatin, collagen, bovine-gammaglobulin, whole dialysed human and rabbit serum, albumin depleted human serum and human skin sections. Molar ratios of coupling of platinum/protein have ranged from 10:1 to 1:100. Negative results were also obtained in RAST tests with the following platinum compounds linked to Sepharose via an amino acid derivative: $\text{K}_2[\text{PtBr}_2(\text{NO}_2)_2]$; $\text{Cs}_2(\text{Pt}(\text{NO}_2)_2\text{Cl}_2)$; $(\text{NH}_4)_2[\text{PtBr}_6]$; *cis*- $\text{Pt}(\text{NH}_2\text{CH}_3)_2\text{Cl}_2$; $[\text{Pt}(\text{NH}_3)_4]\text{Cl}_2$; $(\text{NH}_4)_2[\text{PtCl}_4]$; $(\text{NH}_4)_2[\text{PtCl}_6]$.

The allergenicity as shown by skin tests of the platinum complexes was found to be directly related to both their charge and their overall reactivity. Analysis of their structure and consideration of known chemistry throws some light on the factors underlying this.

Platinum (II) complexes, like other square planar metal systems, react via an associative mechanism with a five-co-ordinate transition state in which both the entering and leaving groups are partially bound to the metal. They obey a general rate law which includes a solvent assisted path. For example,



Rate = $k_1 [\text{PtX}_3\text{Z}] + k_2 [\text{PtX}_3\text{Z}][\text{Y}]$, where k_1 is a first order constant for the solvent controlled path and k_2 is a second order rate constant for the direct reaction with Y. In a good co-ordinating solvent such as water, $k_1 \gg k_2$ and the reactivity of the complex largely depends on the leaving ability of the ligand which is being replaced (Z).

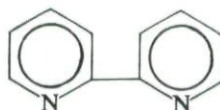
For reactions with proteins *in vivo* we are not in a position to say anything about the incoming group except that it is likely to be the same protein(s) and site(s) which are involved in each case. Thus we must concentrate on the leaving groups in each platinum complex. The kinetic effect of these ligands is not completely clear as yet, but certain general conclusions can be drawn. Three important sets of data are presented in Table 2.

Equations (1) and (2) are probably most relevant as the other co-ordination positions (apart from the leaving group under study) are occupied by an inert trichelating N-donor ligand. Some leaving groups, such as the halogens, are consistently found to be fairly labile and can be classed as reactive, whereas others such as nitro ($-\text{NO}_2^-$) and thiocyanato ($-\text{SCN}$) are more inert and much less reactive. Similarly the platinum amine linkage is very stable and inert to nucleophilic attack while amines are very poor leaving groups.

Table 2. Relative lability $K_2(X)/K_2(N_3)$ of ligands displaced from Pt (II) complexes

(1) $[Pt(dien)X]^+ + py \rightarrow [Pt(dien)(py)]^{2+} + X^-$ (water, 25°C) $Cl > Br > I > N_3 > SCN > NO_2 > CN$ 40 27 12 1.0 0.36 0.056 0.02	(Basolo, Gray & Pearson, 1960)
(2) $[Pt(dien)X]^+ + Y^- \rightarrow [Pt(dien)Y]^+ + X^-$ (water, 30°C) $I \approx Br > Cl > N_3 (> NO_2)$ 73 70 33 1.0 ?	(Belluco <i>et al.</i> , 1966)
(3) $[Pt(bipy)(NO_2)X] + Y^- \rightarrow [Pt(bipy)(NO_2)Y] + X^-$ (Cattalini & Martelli, 1967) (methanol, 25°C) $I > Br > Cl > NO_2 > N_3$ 900 240 140 7 1.0	

dien = diethylenetriamine $H_2N \cdot CH_2 \cdot CH_2 \cdot NH \cdot CH_2 \cdot CH_2 \cdot NH_2$; py = pyridine C_5H_5N ; bipy = 2,2'-bipyridyl



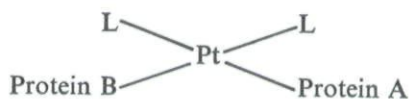
The allergy test results can all be explained on the above kinetic basis. Thus complexes containing strongly bound ligands with poor leaving abilities are not allergenic, presumably due to little or no reaction with proteins (e.g. $[Pt(NH_3)_4]Cl_2$, $K_2[Pt(NO_2)_4][Pt(thiourea)_4]Cl_2$). When reactive halogen groups are present, the complex often elicits some degree of allergic response, with chloro complexes more effective than bromide as would be forecast from equation (1) (the relative reactivities of chloro, bromo and iodo complexes are not consistent for different reasons depending on such factors as the nature of the incoming group). The only exceptions are the neutral diamine species. For most of the others, the activity appears to increase with an increasing number of halide ligands. Table 3 shows that the acid hydrolysis rate constants do not vary greatly for any member of the chloroammine series although the equilibria for the removal of one chloride vary by up to $\times 50$. Generally the rates of substitution in Pt(II) complexes are independent of charge and this makes the inactivity of the neutral species somewhat puzzling as the chloro groups should be sufficiently reactive to cause an effect. Neutral compounds are more lipophilic than charged ones and can penetrate cell membranes more readily.

We can postulate concerning four aspects of the platinum-protein interaction.

Table 3. Acid hydrolysis of Pt (II) chloroammine species

Complex	Equilibrium constants ($M \times 10^5$)		Rate constants ($sec^{-1} \times 10^5$)	
	1st	2nd	1st	2nd
$[PtCl_4]^{2-}$	1500	50	3.9	3.3
$[Pt(NH_3)Cl_3]^-$	<i>cis</i> 1300		0.62	—
	<i>trans</i> 130	4	5.6	—
<i>cis</i> - $[Pt(NH_3)_2Cl_2]$	330	40	2.5	3.3
<i>trans</i> - $[Pt(NH_3)_2Cl_2]$	32	<2	9.8	<5
$[Pt(NH_3)_3Cl]^+$	27		2.6	

(a) As some compounds containing only one reactive group appear to elicit an allergic response, this tends to eliminate protein reactions in which more than one platinum co-ordination position is involved, e.g. protein bridging.



This assumes that several different types of protein interaction are not involved. However $[\text{Pt}(\text{dien})\text{Cl}]\text{Cl}$, which is likely to have low $[\text{PtCl}_4]^{2-}$ contamination, was inactive. The reactions elicited by these compounds were always at the higher concentrations and contamination cannot be eliminated with absolute certainty.

(b) The fact that a number of different platinum compounds can cause allergic effects in people originally sensitized to either $[\text{PtCl}_6]^{2-}$ or $[\text{PtCl}_4]^{2-}$ salts, indicates that the antibodies formed due to the $[\text{PtCl}_6]^{2-}/[\text{PtCl}_4]^{2-}$ carrier conjugate can accommodate different structures within the approximately planar co-ordination around the Pt(II) centre which is presumably bound to a protein or other macromolecule. However, we have not introduced any ligands of large bulk which might be expected to cause steric hindrance in the antigen-antibody compounds. This latter point could be a reason for the inactivity of the $[\text{Pt}(\text{dien})\text{X}]^+$ series, as diethylenetriamine is the bulkiest ligand employed in our tests.

(c) The Pt(IV) and Pt(II) chloro species appear to be equally effective against the same sensitized persons. As has been postulated in the anti-cancer research, it may be that *in vivo* reduction of Pt(IV) $\rightarrow$ Pt(II) occurs. Pt(IV) species are usually rather kinetically inert unless a reaction occurs through a Pt(II) intermediate. However, in a biological area which is sensitive to such small quantities, it is possible (though unlikely) that Pt(IV) reacts in its own right. If this is so, it is surprising that Pt(II), the more kinetically labile species is not a lot more allergenic than $[\text{PtCl}_6]^{2-}$ unless this is due to the fact that the persons tested have become initially sensitized to $[\text{PtCl}_6]^{2-}$ under refinery conditions.

The observations on the nature of the sensitizing material on the one hand and the eliciting agents on the other suggest the following hypotheses.

If chlorine ligands are still attached in the conjugate formed from $[\text{PtCl}_4]^{2-}$ or $[\text{PtCl}_6]^{2-}$ which are known to be sensitizing agents for refinery workers, then the antibodies may have a specific preference for Pt complexes containing Pt-Cl bonds or those containing these after conjugate formation. This may account for the observations that complexes containing several halide ligands are the most successful in eliciting responses in sensitive refinery personnel. Thus both kinetic and antibody specificity factor may be at play in the elicitation of reactions.

The charge on the antibodies could also be of considerable importance. Highly chlorinated species such as $[\text{PtCl}_4]^{2-}$ carry a negative charge and are more likely to give rise to positively charged antibodies, depending on the exact nature of the conjugate, than species containing less or no halide ligands. The latter might well give rise to negatively charged antibodies, being positively charged species. As the workers have been sensitized by inhalation to fully chlorinated species, this might explain their lack of reaction to compounds such as the neutral *cis*- $[\text{Pt}(\text{NH}_3)_2\text{Cl}_2]$ compound. However, it does not eliminate the possibility that the less chlorinated and differently charged compounds which are reasonably reactive may not be sensi-

tizers in their own right and this might be an explanation for the different results reported by Khan *et al.* (1975) for *cis*-[Pt(NH₃)₂Cl₂] given intravenously.

Thus, in sum, the platinum compounds eliciting reactions are confined to a small group of charged compounds containing reactive ligand systems such as chloride and to a lesser extent, bromide. The allergenicity of the complexes is directly related to both their charge and overall reactivity. New compounds could be assessed on the basis of the above.

References

- BASOLO, F., GRAY, H.B. & PEARSON, R.G. (1960) Mechanism of substitution reactions of complex ions. XVII. Rates of reaction of some platinum (II) and palladium (II) complexes with pyridoxine. *Journal of the American Chemical Society*, **82**, 4200.
- BELLUCO, U., ETTORE, R., BASOLO, F., PEARSON, R.G. & TURCO, A. (1966) Activation parameters for some substitution reactions of acidodiethylenetriamineplatinum (II) complexes. *Inorganic Chemistry*, **5**, 591.
- CATTALINI, L. & MARTELLI, M. (1967) Relazione tra reattività di complessi planari del platino e natura del gruppo uscente, in reazioni di sostituzione nucleofila. *Gazzetta Chimica Italiana*, **97**, 498.
- CHERNYAEV, I. (1928) *Izvestiya Instituta Izucheniya Platin i Drugikh Blagorodnykh Metallov*, **6**, 23.
- CLEARE, M.J. (1970) *Studies on platinum metal complexes containing κ -bonding ligands*. Ph.D. Thesis, University of London.
- CLEARE, M.J. (1974) Unpublished results.
- CLEARE, M.J. & HOESCHELE, J.D. (1973) Studies on the anti-tumor activity of Group VIII transition metal complexes. Part. I. *Bioinorganic Chemistry*, **2**, 187.
- CLEARE, M.J. & HOESCHELE, J.D. (1974) Anti-tumour platinum compounds—relationship between structure and activity. *Platinum Metals Review*, **17**, 2.
- DHARA, S.C. (1970) A rapid method for the synthesis of *cis*-[Pt(NH₃)Cl₂]. *Indian Journal of Chemistry*, **8**, 193.
- ELLEMAN, T.S., REISHUS, J.W. & MARTIN, D.S., JR. (1958) *Journal of the American Chemical Society*, **80**, 536.
- ERICKSON, L.E., McDONALD, J.W., HOWE, J.K. & CLOW, R.P. (1968) Proton magnetic resonance studies of amino acid complexes of platinum (II). I. Synthesis, spectral interpretation and conformational implications. *Journal of the American Chemical Society*, **90**, 6371.
- FREEDMAN, S.O. & KRUPY, J. (1968) Respiratory allergy caused by platinum salts. *Journal of Allergy*, **42**, 233.
- HUGHES, E.G. Personal communication.
- HUNTER, D., MILTON, R. & PERRY, K.M.A. (1945) Asthma caused by complex salts of platinum. *British Journal of Industrial Medicine*, **2**, 92.
- ISHIZAKA, K. & ISHIZAKA, T. (1971) Mechanisms of reaginic hypersensitivity: a review. *Clinical Allergy*, **1**, 9.
- KHAN, A., HILL, J.W., GRATER, W., LOEB, E., MACHALLAN, A. & HILL, N. (1975) Atopic hypersensitivity to *cis*-dichlorodiammine-platinum (II) and other platinum complexes. *Cancer Research*, **35**, 2766.
- KAUFFMAN, G.B. & COWAN, D.O. (1963) *Cis* and *trans*-dichlorodiammine-platinum (II). In: *Inorganic Synthesis* (Ed. by J. Kleinberg), **7**, 242. McGraw-Hill, New York.
- KELLER, R.N. (1946) Tetrammine platinum (II) chloride. *Inorganic Synthesis*, **2**, 250.
- KURNAKOV, N.S. (1893) On complex metallic bases. *Journal of the Russian Physical Chemical Society*, **25**, 565.
- KURNAKOV, N.S. (1894) *Journal of the Russian Physical Chemical Society*, **50**, 480. (In Russian.)
- KURNAKOV, N.S. (1894) Ueber complexe metallbasen. *Journal für Praktische Chemie*, **50**, 481.
- LEVENE, G.M. & CALNAN, C.D. (1971) Platinum sensitivity and treatment of specific hypersensitization. *Clinical Allergy*, **1**, 75.
- MATTHIEU, J.P. & CORNEVIN, S. (1939) Recherches sur les complexes de werner spectres raman de complexes tetraco-ordinés et hexaco-ordinés. (I) Chlorure, cyanures et nitrites. *Journal de Chimie Physique et de Physico-chimie Biologique*, **36**, 209.

- PARROT, J.-L., HEBERT, R., SAINDELL, A. & RUFF, F. (1969) Platinum and platinosis. Allergy and histamine release due to some platinum salts. *Archives of Environmental Health*, **19**, 685.
- PEPYS, J., PICKERING, C.A.C. & HUGHES, E.G. (1972) Asthma due to inhaled chemical agents—complex salts of platinum. *Clinical Allergy*, **2**, 391.
- PRESSMAN, D. (1971) The nature of antigen and antibody combining regions. In: *Immunological Diseases* (I) (Ed. by M. Samter), p. 11. Little, Brown & Co., Boston.
- ROBERTS, A. (1951) Platinosis—a five year study of the effects of soluble platinum salts on employees in a platinum laboratory and refinery. *Archives of Industrial Health & Occupational Hygiene*, **4**, 549.
- ROSENBERG, B., VANCAMP, L., TROSKO, J.E. & MANSOUR, J.H. (1969) Platinum compounds: A new class of potent antitumour agents. *Nature*, **222**, 385.
- SQUIRE, J.R. (1952) Tissue reactions to protein sensitisation. *British Medical Journal*, **1**, 1.
- VON HOFF, D.D., SLAVIK, M. & MUGGIA, F.M. (1976) Allergic reactions to cis-platinum. *Lancet*, **i**, 90.
- WATT, G.W. & CUDE, W.A. (1968) Diethylenetriamine complexes of platinum (II) halides. *Inorganic Chemistry*, **7**, 335.
- WEBSTER, I. Personal communication.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.