IgE antibody responses to platinum group metals: a large scale refinery survey

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ABSTRACT All 306 South African platinum refinery workers (116 white, 190 coloured) accepted for employment on grounds of absence of evidence of atopy were investigated using the skin prick test and RAST to detect sensitivity to platinum, palladium, and rhodium salts. RAST studies were made for these, together with HSA and DNP-HSA RAST. Of the 306 workers, 38 had a positive skin prick test to the platinum halide salts; of these, one gave a positive reaction to the palladium salt and six to the rhodium salt. There were no isolated positives to the rhodium and palladium halide salts. Total IgE levels were raised in 24 of the 38 (63%) platinum salt prick test positive workers compared with only 43 of the 268 (16%) prick test negative group (p < 0·001). Positive RASTs were obtained in 62% of those with positive skin tests to the platinum salts. Four of the six giving positive rhodium salt skin tests gave a positive RAST to rhodium salt. Of these, two gave positive RASTS to HSA and all four to DNP-HSA. The palladium salt RAST was negative in the single skin test reactor. In the platinum salt skin test positive group a raised HSA RAST was obtained in 10·5% compared with only 2·5% in the skin negative group. Twenty one per cent of the platinum salt skin positive group had a raised RAST score to DNP-HSA with only 3·5% (4/116) in the skin test negative group, of whom three also had a raised HSA RAST. The latter findings are suggestive of IgE antibody production to new antigenic determinants in HSA produced by conjugation with the platinum salts.

Ammonium tetrachloroplatinite II [$(NH_4)_2PtCl_4$] ammonium hexachloroplatinate IV [$(NH_4)_2PtCl_6$] have been implicated as the main occupational sensitising agents among platinum refinery workers and both are capable of inducing and eliciting type 1 hypersensitivity in exposed workers.[1-3] Little is known, however, concerning the sensitising capacity or indeed the cross reactivity between the halide salts of platinum and the other platinum group metal salts such as palladium and rhodium. During the time at work, platinum refinery process workers are known to be exposed to comparable levels of the salts of palladium and rhodium.

There are some reports of sensitivity to palladium and palladium salts.[4-6] Most of the reports, however, are concerned with contact dermatitis, a type IV reaction, whereas allergy to the platinum halide salts is predominantly a type I reaction. Reports of rhodium salt sensitisation per se are much less common (G M Levene, personal communication).

The sensitising and eliciting capacity of these metal salts was studied in a large platinum refinery where workers with positive skin prick tests to the salts of platinum and other platinum group metal salts were found. It should be noted, however, that in no cases were positive reactions found in the absence of a positive platinum halide salt skin test.

In occupational allergic disease there may be an appreciable cross reactivity between closely related analogues of the principle sensitising/eliciting agent. This is especially true of the acid anhydrides, phthalic, trimellitic, and tetrachlorophthalic[78] and the isocyanates, toluene diisocyanate and its analogues p-TMI, MDI, and HDI.[9]

It is also possible that antibodies directed against the probable carrier molecule, human serum albumin (HSA), HSA in partially denatured form, or both, may be induced by the effects of the hapten conjugation in vivo. This has been shown in other compounds where new antigenic determinants (NADS) may be formed after hapten binding with TDI.[10] Furthermore, in subjects sensitive to nickel antibodies have been directed against Ni-HSA fragments.[11 12]

The aims of this study, therefore, were to

investigate the prevalence of platinum salt allergy, and of antibody directed against other platinum group metal salts to determine the presence of antibody to HSA and antigenically denatured HSA-DNP, to study their relations, and to correlate these findings with IgE levels.

Materials and methods

SUBJECTS INVESTIGATED

Sera were collected from 306 South African platinum refinery workers (116 white, 190 coloured) over three days. This represented 100% of the work force, all of whom had a negative history of allergic symptoms to common environmental allergens in their pre-employment medical screening. (Atopic personnel are not employed because of their greater predisposition to develop platinum salt hypersensitivity.)

Eight cord blood sera were used for minimum non-specific RAST binding (mean total IgE 0·5 IU/ml).

Twenty sera from normal non-atopic white British blood donors (mean total IgE 30·4, range 2–158 IU/ml) were used for comparative purposes only in assessing total IgE levels.

Ten patients with Hodgkin's disease with raised total serum IgE levels (between 1750 and 22 500 IU/ml) with no known specificity were used to determine the effects of high total IgE in the individual RAST systems.

SKIN TESTS

All 306 subjects were skin prick tested on the washed volar surface of the arm with ammonium hexachloroplatinate IV, sodium hexachloroplatinate, and sodium tetrachloroplatinite,[3] dissolved freshly in Coca's fluid (5·0 g NaCl, 4·0 g phenol, and 2·75 g NaH CO$_3$ in 100 ml distilled water). Tenfold dilutions were made from 10^{-3} to 10^{-9} g/ml. A positive reaction was taken to be greater than a 2 mm weal. All were also tested with the corresponding salts of palladium, rhodium, iridium, and ruthenium.

PLATINUM GROUP METAL SALTS (PGM)

The platinum group metal salts used in both skin prick test and in conjugate preparation were kindly supplied by Johnson Matthey Chemicals, Royston. All were of high grade spectroscopic purity.

TOTAL SERUM IgE DETERMINATIONS

IgE levels were measured by a direct solid phase radioimmunoassay (phadebas IgE PRIST, Pharmacia).

PLATINUM GROUP METAL SALT (PGM) RAST
Preparation of sepharose HSA-PGM

The solid phase preparations in all platinum group metal salt RAST systems used the method of Cromwell *et al.*[13] Solid phase preparations consisted of 50 ml packed volume of the sepharose-HSA complex to which was added 150 ml, 0·33 mM of either ammonium tetrachloroplatinite II, ammonium tetrachloropallidite II, or ammonium aquopentachlororhodite III in PBS without azide. The suspensions were mixed for three hours at room temperature followed by 18 hours at 4°C. The solid phases were washed with 2 litre quantities of phosphate buffered saline and stored as a 1:4 suspension in phosphate buffered saline (0·02% sodium azide and 0·5% normal horse serum (NHS).

Sepharose—HSA preparation

Coupling HSA to CNBr activated sepharose used the method of Cromwell *et al* with the exception that 5 mg HSA (Sigma V) was coupled to 1 ml packed volume rather than 10 mg/ml used by Cromwell *et al.*[13]

Sepharose HSA-DNP

One hundred milligrams of HSA were dissolved in 5 ml, 0·15 M potassium carbonate, to which was added 20 mg dinitrobenzene sulphonate, sodium salt, and mixed for 18 hours at 4°C. The resulting conjugate was passed through a Sephadex G25 column. The conjugates were diluted 1:20 and the hapten loading on the carrier protein determined spectrophotometrically, at 280 nm and 360 nm, allowing for DNP interference with the protein OD at 280 nm.[14] The conjugate contained 6·1 DNP/protein molecule. The protein-DNP conjugate was then coupled at 10 mg/ml to a Sepharose solid phase after the method of Cromwell *et al.*[13]

RAST PROCEDURES

Serum samples were tested blind in duplicate on one occasion by the addition of 50 μl of neat serum to 100 μl 1:4 suspension of freshly washed solid phase in polystyrene tubes (Seward GW4). Tubes were sealed with plate sealing tape (Selotape 3M) and incubated for 18 hours at room temperature with horizontal shaking. The solid phase was washed three times with saline (0·9% NaCl, 0·02% NaN$_3$, 0·5% Tween 20, 0·5% normal horse serum) by resuspension and centrifugation. Then 100 μl ^{125}I labelled human anti-IgE (Pharmacia) was added and incubated for a further 18 hours at room temperature. Tubes were washed three times, as before, and counted for one minute on a gamma counter (Hydragamma, Innotron).

Sera for each RAST system were tested in one assay to eliminate interassay variation. Sera from cord blood, normal donors, patients with Hodgkin's

disease, and known platinum positive controls were included in each assay. This allowed the determination of minimum baseline binding, maximum non-specific uptake, and assay reproducibility respectively. Sera giving RAST scores at or greater than unity in the platinum RAST were selected for further study.

Results

SKIN PRICK TESTS

All 306 subjects were skin prick tested to the salts of platinum palladium, rhodium iridium, and ruthenium and 38 gave reproducible positive Type 1 reactions to ammonium hexachloroplatinate, sodium hexachloroplatinate, and sodium tetrachloroplatinite at 10^{-3} g/ml or less. Of these, 28 were positive to the Pt salts only, one to halide salts of Pt and Pd, one to

halide salts of Pt and Ru, two to halide salts of Pt and Ir, one to halide salts of Pt and Rh, one to halide salts of Pt, Ru, Ir, and Rh, and four to halide salts of Pt, Rh, and Ir. The remaining 268 were skin test negative. The 20 blood donors were not tested. None was atopic or had worked in a refinery or with precious metals.

TOTAL IgE

Figure 1 shows that 24 of the 38 skin test positive group had raised total serum IgE levels relative to the blood donor pool, with anthmetic mean values of 442 IU/ml (SD 563 range 25–2500 IU/ml) for the refinery population and 30·1 IU/ml (SD 43·3 range 5–150 IU/ml) for the control pool respectively.

The reason for the raised IgE levels in the platinum salt skin test positive group is not known. There is evidence in animals that IgE levels, both total and specific, are raised in adjuvant *(B pertussis)* primed animals with chronic exposure to platinum salts, as
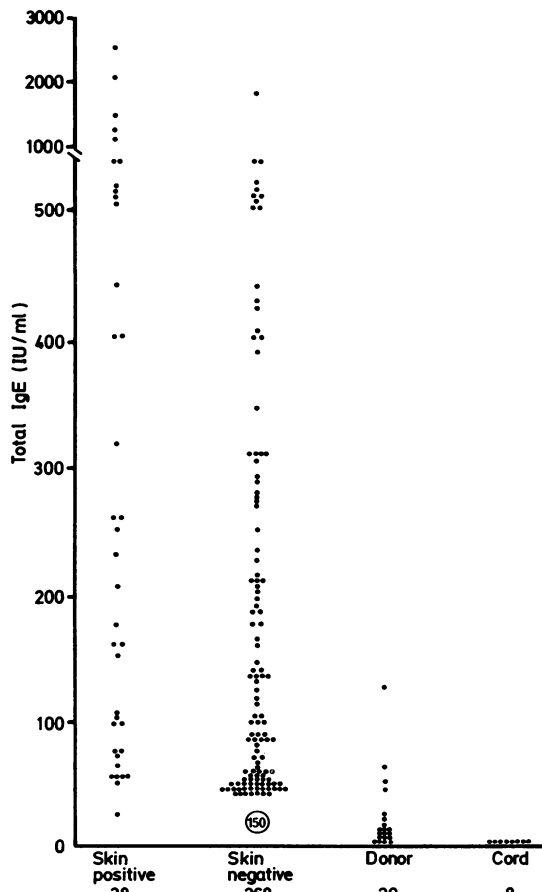

Fig 1 *Total serum IgE in platinum salt skin test positive and skin test negative refinery workers, non-atopic blood donors, and cord blood sera.*
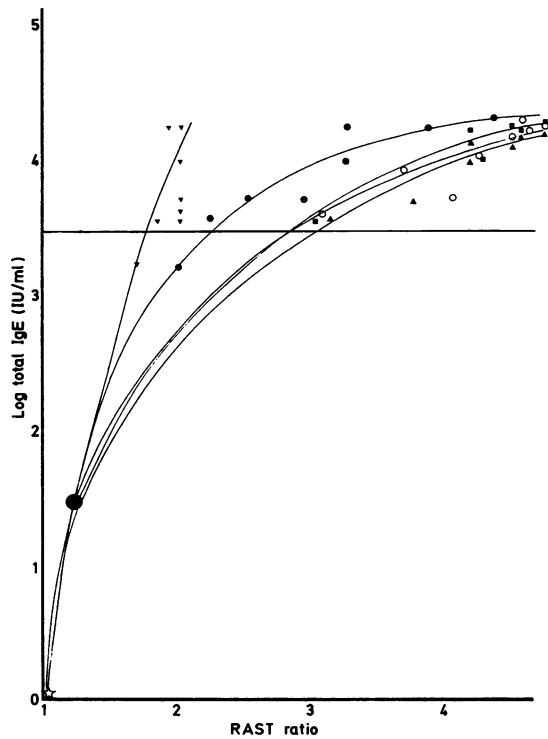

Fig 2 *Non-specific interference of pooled cord blood (☆), pooled non-atopic donor sera (●) and eight Hodgkin's patients' sera with differing levels of serum IgE (1750–22,500 iu/ml) in the five RAST systems. (●) platinum, (▼) palladium, (■) rhodium, (▲) HSA, and (○) DNP-HSA. Solid horizontal line at 3000 iu/ml indicates upper limit of total IgE interference by test sera in various systems.*

opposed to the effects of adjuvant alone.[19] It has also been found that total IgE levels are raised in platinum sensitive workers as compared with non-sensitive workers.[13]

NON-SPECIFIC IgE UPTAKE

Eight cord sera were tested in all assays to determine the lowest level of non-specific uptake. As a result of these findings subsequent readings were expressed as a RAST ratio relative to the cord levels:

$$\text{RAST ratio} = \frac{\text{Sample cpm} - \text{background cpm}}{\text{Mean cord cpm} - \text{background cpm}}.$$
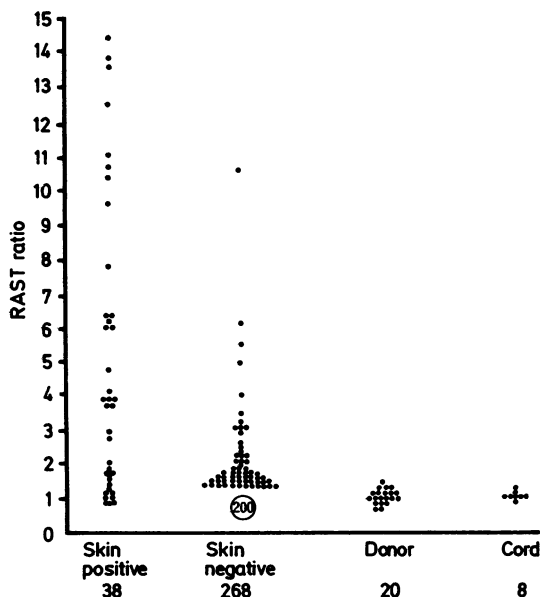

Fig 3   *Platinum RAST in platinum salt skin test positive and negative subjects, non-atopic donor sera, and cord blood.*
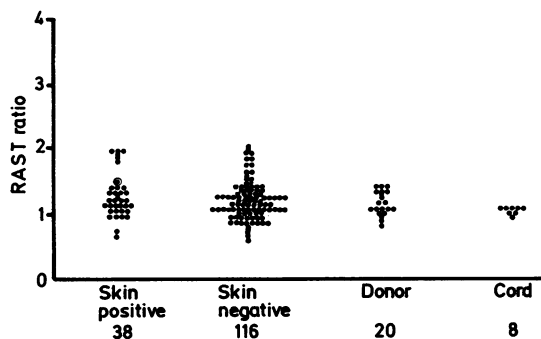

Fig 4   *Palladium RAST in platinum salt skin test positive and negative subjects, non-atopic donor sera, and cord blood. (○) denotes palladium salt skin test positive subject.*

Figure 2 shows the RAST ratios for the cord pool, blood donor pool, and the individual patients with Hodgkin's disease. Pooled cord sera by definition gave a value of 1 unit. Pooled donor sera gave a mean value of 1·2 units in all systems and those of the patients with total IgE levels above 10 000 IU/ml gave values of 4·0 units or more.

None of the total IgE levels for the refinery group was greater than 2500 IU/ml, thus an arbitrary designated cut off ratio for non-specific binding at 3000 IU/ml was taken for each RAST system to exclude any possible interference due to high IgE levels. The ratios taken as probably positive for each system was as follows: platinum 2·1, rhodium 2·6, palladium 1·8, HSA 3·0, and DNP-HSA 2·8.

PLATINUM SALT RAST (fig 3)

All 306 sera were assayed blind for platinum salt antibody levels and subsequently separated into skin test positive and skin test negative groups. Of the 38 skin positive group, 23 (62%) had ratios greater than the cut off point of 2·1 units, many sera giving ratios 10-fold greater than the cord levels. By contrast only 16 (6%) of the 268 platinum skin test negative group had ratios greater than 2·1 units. The donor control pool ratio was 1·2.

PALLADIUM SALT RAST (fig 4)

The RAST ratios found in the 38 platinum salt skin test positive group were no different from the selected 116 skin test negative subjects whose RAST ratios were equal to or greater than unity. No values greater than 2 units were observed; this correlated well with the presence of only one skin test positive reaction to the palladium salt. This worker gave a 2 mm weal at 10^{-5} g/ml and a RAST ratio of 1·35 units. Donor blood samples gave values close to unity.

RHODIUM RAST (fig 5)

Of the 38 platinum salt skin test positive subjects, six also had positive reactions to the rhodium salt. Four of the six also had RAST scores greater than 2·6; only five of the 116 platinum salt skin test negative (4%) had values greater than this.

HSA RAST (fig 6)

Taking the ratio of 3 as the probable criterion of positivity, the incidence of significant binding to the HSA solid phase was low in all groups. Four (10·5%) of the 38 platinum salt skin test positive group and only three (2·5%) of the 116 platinum salt negative group gave positive results.

DNP-HSA RAST (fig 7)

The conjugation of DNP to HSA to form antigenically denatured HSA produced greater numbers

of positive results in the platinum salt sensitive group, eight of the 38 (21%) having ratios greater than the non-specific uptake level of 2·8. Of these eight, four had positive RASTs to HSA. Positive RASTs to the rhodium salt were obtained in four of this group, and of these, two had positive RASTs to HSA and four to DNP-HSA. Positive RASTs were obtained with the rhodium salts only with sera from platinum salt positive subjects. In the platinum salt skin test negative group only four of the 116 (3·4%) had positive RASTs to DNP-HSA and three of these gave positive HSA RASTs. All four platinum salt skin test positive subjects with raised HSA Rasts also had positive DNP-HSA RAST scores. Four of the eight platinum salt sensitive group with positive DNP-HSA RAST also had positive rhodium salt RAST.

CORRELATION OF TOTAL SERUM IgE LEVELS AND RAST RATIO

The sera of the patients with Hodgkin's disease with greatly raised total IgE levels gave positive RAST scores in all five systems. Examination of the RAST scores and total IgE levels in the refinery workers using Spearman's rank correlation test gave the following results.

Total IgE v platinum-HSA RAST, r = 0·52
Total IgE v rhodium-HSA RAST, r = 0·31
Total IgE palladium-HSA RAST, r = 0·18
Total IgE v HSA RAST, r = 0·36
Total IgE v DNP-HSA RAST, r = 0·34

It may be concluded, therefore, that whereas greatly raised total IgE levels, as found in the Hodgkin's sera, significantly increase RAST scores, this is not the case with the RASTs for palladium, rhodium, HSA, and DNP-HSA. With regard to the total IgE Pt-HSA comparisons there was some correlation. Pt-HSA RASTs (fig 3), however, show much higher RAST values for many sera with total IgE levels not exceeding 2500 units as compared with the values for the Hodgkin's sera when many sera were in excess of 10 000 units.
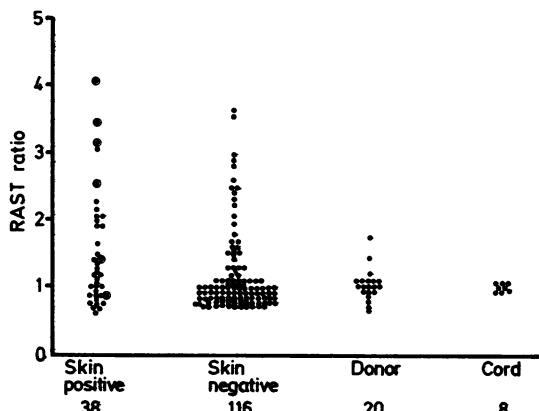

Fig 5 *Rhodium RAST in platinum salt skin test positive and negative subjects, non-atopic donor sera, and cord blood. (○) denotes rhodium salt skin test positive subjects.*
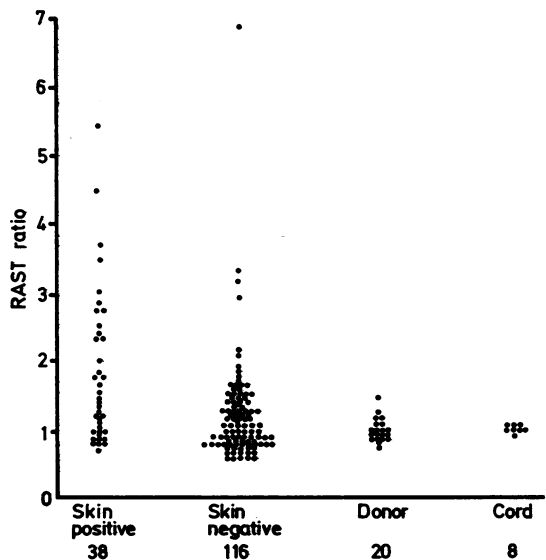

Fig 6 *Human serum albumin (HSA) RAST in platinum salt skin test positive and negative subjects, non-atopic donor sera, and cord blood.*
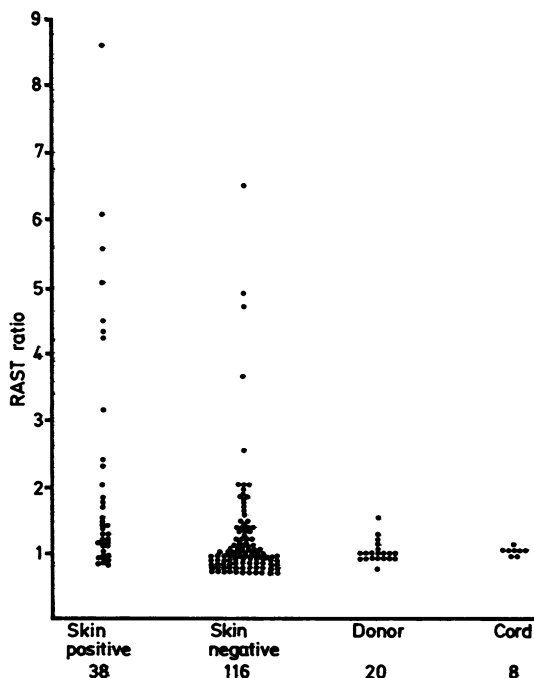

Fig 7 *DNP-HSA RAST in platinum salt skin test positive and negative subjects, non-atopic donor sera, and cord blood.*

Discussion

The potent allergenicity of the halide salts of platinum in refinery conditions is well documented and the causal agents have been identified as the divalent and tetravalent platinum compounds possessing reactive halide ligands—namely, ammonium tetrachloroplatinite and ammonium hexachloroplatinate.[3] Their mode of action is thought to be by conjugation with sulphydryl containing groups within proteins, thus forming immunogenic complexes. It might be expected that other group VIII metal salts—for example, palladium and rhodium—which possess similar physicochemical characteristics in terms of their size, charge, and protein reactivity would be capable of firm conjugation with proteins in vivo to form stable complexes, and that these conjugates in vivo should be capable of inducing antibody formation and hypersensitivity. Past studies, however, have shown that other group VIII metal salts are not potent allergens. The present study of platinum salt positive prick tests and positive RASTs shows moderately good concordance, and also shows that the other platinum group metal salts are poor sensitisers as shown by skin prick test (at non-histamine releasing concentrations) or the RAST. Of the 306 workers, only six had positive skin tests to the rhodium salt and only one had a positive prick test to the palladium salt. Interestingly, there were no cases of positivity to these salts in the absence of a platinum salt skin test reaction.

There is some evidence to suggest that in some workers limited cross reactivity between the salts of platinum and palladium salts might be present in sensitised workers. This evidence comprises: (1) the presence of palladium salt skin prick positivity which has been noted in platinum salt sensitised workers, with no reports of isolated type 1 hypersensitivity to palladium salts; (2) PCA reactions in the monkey which have been obtained to the palladium salt only with the sera of platinum sensitive workers; and (3) PCA cross absorption studies in the monkey (R D Murdoch, in preparation) which have failed to detect specific palladium salt antibodies when the platinum salt antibody activity has been absorbed, and that the palladium salts can also absorb though not completely the platinum salt activity. RAST inhibition studies (R D Murdoch, personal communication) using the same subject as in (3) corroborate these findings.

In tests for antibodies against new antigenic determinants in HSA, which is a probable carrier protein by virtue of its high concentration in body fluids, the incidence of significant RAST binding in the HSA RAST system showed low levels, with only 2·5% of the 116 platinum skin test negative subjects tested being positive, whereas 10·5% (4/38) of the platinum sensitive subjects were positive. The presence of IgE antibodies against denatured HSA in 21% of those possessing IgE antibody against the platinum salts, compared with only 3·4% in the skin test negative group, suggests that the HSA is behaving as a partial antigenic determinant in its denatured form.

In both the DNP and the platinum salt RAST solid phase the degree of haptenic substitution was 6·1 and 8. Thus with similar substitutions the question arises as to where the haptens are conjugated. DNP-HSA was taken, arbitrarily, as an example of denatured HSA in tests for antibodies to new antigenic determinants on HSA. DNP will preferentially bind to lysine residues and terminal amino groups, whereas the platinum halide salts are preferentially bound to the sulphydryl groups of methionine, cysteine, and histidine. Those that are accessible are, however, limited to fewer than the number of bound platinum molecules and the possibility of the highly charged platinum complex penetrating the hydrophobic interior of the HSA is questionable; this suggests that binding must occur elsewhere. It is not known, however, whether the conformational changes brought about by DNP and the platinum salts would produce sufficiently similar changes to allow binding of closely related antibody. The ideal control would be a heavy metal salt-HSA interaction, but it was thought that a denatured HSA unrelated to the effects of such salts was preferable. The positive RASTs to DNP-HSA would support the assumption that HSA is acting as a carrier molecule for the haptenic platinum salts and that a denatured site is produced within the HSA molecule capable of binding specific IgE antibody. This denaturation may be at sites common to the platinum, palladium, and rhodium salts and to DNP, accounting for the large numbers of positive responses. The possibility of specific hypersensitivity to the other platinum group metals cannot be excluded, though the evidence shows that this is unlikely. Cross reactivity based on antibodies to denatured HSA, which could readily be produced by these metals, provides an alternative explanation for reactions to them occurring in the platinum sensitive subjects.

High total serum IgE levels are known to interfere with all RAST systems so there are always problems of deciding the criteria for a positive result. Thus by choosing a RAST cut off point greater than any effect which could be caused by non-specific high total IgE interference, the test is more useful for screening large numbers of sera without the need for dilution of serum samples. Diluting the sera 1/10 is known to result in 60% reduction in platinum salt RAST binding (unpublished data). In recent investigations the presence of antibody to other platinum group metal salts, in particular to palladium, has been reported,[16]

but could not be confirmed in a later study with the same sera (RD Murdoch, unpublished data).

Soviet investigations have reported type 1 hypersensitivity in rodents and rabbit to most group VIII metal salts and to other heavy transition metal salts.[17-19] Many of their protocols have been repeated in attempts to induce or to elicit type 1 reactions without success.[15 20] At these specified doses for eliciting reactions, the histamine liberating properties of the platinum salts have played a major pharmacological part.[21]

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