

Specific IgE antibodies to platinum salts in sensitized workers

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Summary

A RAST has been developed for the measurement of IgE antibodies specific to platinum chloride complexes in sensitized workers. Human serum albumin covalently linked to Sepharose beads by the cyanogen bromide method was reacted with ammonium tetrachloroplatinite (II) $(\text{NH}_4)_2\text{PtCl}_4$. This conjugate was more suitable for the RAST, than conjugates of HSA and the platinum salt prepared in solution and then linked to the activated Sepharose, showing better sensitivity and giving lower levels of non-specific uptake of IgE from sera of non-exposed subjects with high total levels of IgE, e.g. allergic bronchopulmonary aspergillotics.

Introduction

Sensitivity to halide complexes of platinum is encountered in workers involved with platinum refining processes, and manifests itself in most cases by rhinitis, conjunctivitis and asthma, and occasionally urticaria and contact dermatitis (Hunter, Milton & Perry, 1945; Pepys, Pickering & Hughes, 1972; Pickering, 1972). Typical type I skin, nasal and bronchial reactions have been demonstrated Pepys *et al.*, 1972), suggesting that IgE antibodies may be implicated.

Passive transfer of sensitivity with a single serum from a sensitized inorganic chemist was demonstrated in man by the P-K test (Freedman & Krupey, 1968) and with a group of six sera in both man and the monkey (Pepys *et al.*, 1979) supporting the assumption of a type I reaction mediated by specific IgE antibody.

We have been unable to demonstrate until recently the presence of specific IgE antibodies to the haptenic platinum salts with *in vitro* tests. This has been hindered by the problems of finding an appropriate carrier or a method of preparation to minimize non-specific reactions in non-exposed subjects. Ammonium hexachloroplatinate (IV) and tetrachloroplatinite (II), the salts most commonly implicated in platinum hypersensitivity reactions, have molecular weights of 444 and 373 daltons

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respectively, and molecular sizes within the hapten size range. Conjugates of platinum salts with human serum albumin (HSA) were found, by intracutaneous tests, to give positive reactions in passive transfer testing (Freedman & Krupey, 1968). Similar conjugates with HSA, transferrin and ovalbumin were not active in skin prick tests, nor were they suitable for the RAST (Cleare *et al.*, 1976), being unable to distinguish between sensitized workers and some non-exposed subjects.

We report here on the development of a radioimmunoassay for specific IgE antibody in sensitized workers to the platinum salts.

Materials and methods

In the present study ammonium tetrachlorplatinit (II) was used throughout.

Sera

(a) Sera were collected from forty-seven platinum refinery workers, twenty-six sensitive and twenty-one non-sensitive to prick tests with the platinum salt and with various lengths of employment and exposure. (b) Eight cord blood sera were used as controls. (c) Two groups of six and twelve skin test-negative non-exposed controls were tested on separate occasions, together with the two groups of sera in (a) above. (d) Sera containing raised levels of total IgE were taken from ten patients with allergic bronchopulmonary aspergillosis. (e) A group of fifty-four blood bank sera from healthy subjects were used to establish a 'normal range' for total IgE.

Skin prick tests

These were made with ammonium hexachlorplatinate (IV) and tetrachlorplatinit (II) dissolved in Coca's fluid (5.0 g NaCl, 4.0 g phenol and 2.75 g NaHCO₃ to 1.0 litre with distilled water) in ten-fold dilutions from 10⁻³ to 10⁻⁹ g/ml (Cleare *et al.*, 1976).

Preparation of RAST conjugates

Cyanogen bromide activation of Sepharose. Sepharose 4B (Pharmacia, London) was activated with cyanogen bromide using a modification of the method of March, Parikh & Cuatrecasas (1974). 50 ml packed volume of Sepharose was washed with distilled water to remove preservative, made up to 100 ml with water and mixed with 100 ml 2 M sodium carbonate. A solution of 10.0 g cyanogen bromide (Koch-Light, Colnbrook, England) in 5.0 ml methyl cyanide was added, and the suspension stirred vigorously for 2 min. The Sepharose was recovered by filtration and washed successively with 1.0 litre vols of 0.1 M sodium bicarbonate, pH 9.5, water and 0.2 M sodium bicarbonate, pH 8.5.

Coupling of protein to activated Sepharose. 50 ml of activated Sepharose was suspended in a solution of 500 mg HSA (Behringwerke) in 50 ml 0.2 M sodium bicarbonate, pH 8.5. This was mixed for 4 hr at room temperature and then for 20 hr at 4°C. Glycine was then added to a final concentration of 0.2 M, and after mixing for 4 hr at room temperature the Sepharose was washed successively with phosphate-buffered saline (PBS) (pH 7.3), 0.1 M sodium acetate (pH 4.0), PBS, 0.1 M sodium bicarbonate (pH 9.5) and PBS. In excess of 90% of the protein remained coupled to the Sepharose as determined by the Lowry method after alkaline hydrolysis (Cromwell, 1975). Preparations were stored in PBS with 0.1% sodium azide at 4°C.

Preparation of (a) Sepharose HSA-Pt and (b) Sepharose-HSA Pt conjugates.

(a) 70 mg HSA was dissolved in 5 ml PBS, without azide, and placed in a dialysis sac (Visking tubing). The protein solution was dialysed against a solution of ammonium tetrachloroplatinite (II) in PBS in a screw-top bottle at 4°C on a rock and roll mixer (Luckhams, Burgess Hill, U.K.) for 48 hr. Resulting conjugates were coupled to activated Sepharose (Fig. 1).

(b) 50 ml packed volume of the Sepharose-HSA complex was suspended in 150 ml 0.33 mM ammonium tetrachloroplatinite in PBS without azide. The suspension was mixed for 4 hr at room temperature and then 20 hr at 4°C. The Sepharose was recovered by filtration, washed with 2.0 litre PBS and stored as a 1:4 suspension in assay buffer at 4°C (Fig. 2).

Pt conjugates using methionine and human gamma globulin. Conjugates of Sepharose and methionine and human gamma globulin were made as for the HSA above.

Radioallergosorbent test (RAST) (Fig. 3)

Serum samples were assayed in duplicate by adding 50 μ l samples to polystyrene tubes (12.5 $\times$ 75.0 mm, Seward, London) and mixing with 100 μ l of a 1:4 suspension of platinum salt-HSA-Sepharose conjugates in PBS plus 0.3% HSA. Tubes were capped and incubated at room temperature for 18 hr on a horizontal shaker. The solid phase was washed three times by resuspension in PBS followed by centrifugation (500 g, 10 min) and aspiration of the supernatant. Anti-human IgE (Fc-specific) labelled with ^{125} I by the chloramine-T method (Hunter & Greenwood, 1962) was added to each tube—50 μ l containing 10⁵ct/min. The tubes were capped and shaken overnight

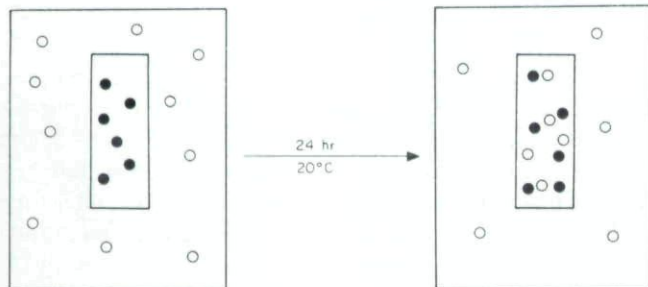


Fig. 1. Conjugation of protein and ammonium tetrachloroplatinite. (●) Protein molecules; (○) ammonium tetrachloroplatinite.

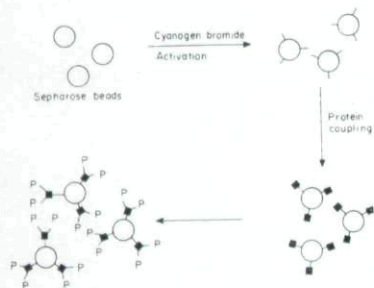


Fig. 2. Coupling of protein to sepharose beads and conjugation with ammonium tetrachloroplatinite. (■) Protein; (P) (NH₄)₂PtCl₄.

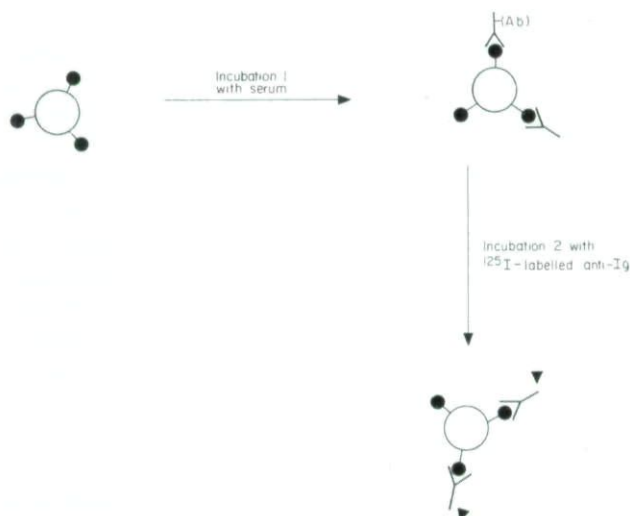


Fig. 3. Radioimmunoassay for antibodies specific for 'platinum' salts. (●) Platinum-protein conjugate; (○) Sepharose bead; (▲) ¹²⁵I-labelled anti-immunoglobulin; (Ab) specific antibody.

before washing the solid phase five times with PBS plus 0.5% Tween 20. Tubes were counted for 60 sec in an LKB 80000 automatic γ -counter, Cord, sera and sera from atopic and non-atopic individuals, were included as controls in each assay. RAST counts greater than the mean plus two standard deviations of those in the control group were taken as positive.

Measurement of total IgE

A solid phase radioimmunoassay was used. Cyanogen bromide activated Sepharose 4B was reacted with ϵ -chain-specific anti-IgE (Behringwerke) in the ratio 12.5 ml packed volume Sepharose to 1.0 ml antiserum. 100 μ l of a 1:4 dilution of the solid phase in PBS plus 0.3% HSA was incubated with 50 μ l of serum for 18 hr. After three washes with PBS an excess of ¹²⁵I-labelled Fc-specific goat anti-human IgE was added, and after a further 18 hr incubation and five washes with PBS plus 0.5% Tween 20, the uptake of ¹²⁵I-labelled antibody was measured in a γ -counter. Standard curves were constructed using dilutions of British Working Standard 75/502 and a laboratory standard. The 'normal range' ascertained by assaying fifty-four normal blood bank sera was 2–240 iu IgE per ml.

Anti-IgE

Goat anti-human IgE (Fc) antibody, purified by affinity chromatography and judged to be 50% pure, was a gift from Dr C. E. Arbesman, Buffalo, New York.

Results

Skin prick tests

Of the forty-seven platinum refinery workers, twenty-six gave positive skin prick tests to ammonium tetrachloroplatinite (II) at concentrations of 10^{-3} g/ml or less, and the remaining twenty-one were skin test-negative.

RAST values in workers and controls

Fig. 4 shows that skin prick test-positive and -negative groups were significantly different ($P < 0.001$) by RAST (Student's t -test after logarithmic transformation of the data). Twenty-one of the twenty-six skin test-positive group gave RAST counts greater than 2 s.d. above the mean of the skin test-negative group, but only seventeen of them when compared with the control group. The mean RAST counts for the skin test-negative and control groups were 1713 ± 17.5 and 1917 ± 211 ct/min respectively. Repetition of the test with a different batch of immunosorbent and a larger control group of twelve sera gave comparable results. The degree of skin prick test sensitivity of the skin test-positive/RAST-negative group was no different from that of the skin test-positive/RAST-positive group.

Control RAST with cord blood sera and sera with raised total IgE values

The RAST (ct/min) obtained with the eight cord sera showed the lowest counts with the conjugates having the lowest Pt content (Table 1). The ratios of ct/min between the sera from the patients with allergic bronchopulmonary aspergillosis and the cord blood sera for the two preparations of Sepharose-HSA Pt and the Sepharose HSA-Pt are shown in Table 1. These were greater than unity with the Sepharose-HSA Pt conjugates. Ratios closer to unity were obtained with the Sepharose HSA-Pt conjugate except for two of the aspergillosis sera which had total IgE values of 5000 and 9000 iu/ml respectively.

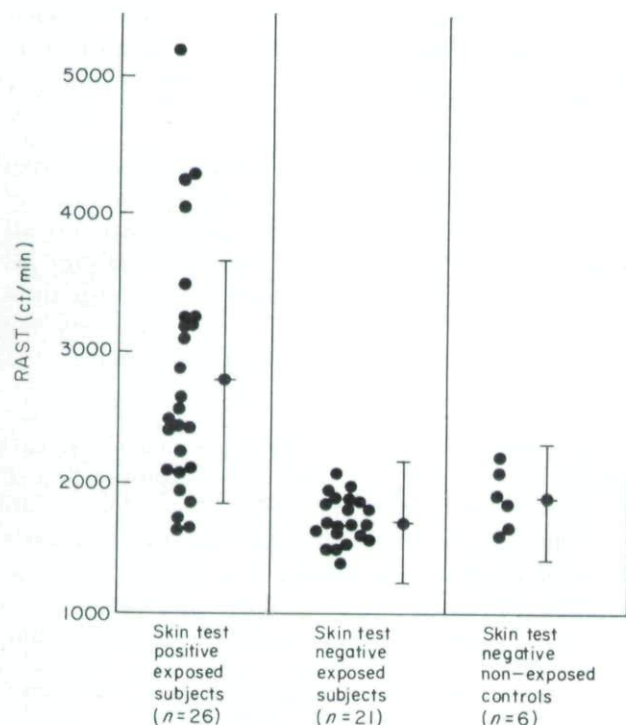


Fig. 4. RAST with Sepharose HSA- $(\text{NH}_4)_2\text{PtCl}_4$ conjugate (Mean $\pm$ 2 s.d.).

Table 1. RAST values platinum salt conjugates/Total IgE

Compound	RAST ratio (serum: cord blood)		
(NH ₄) ₂ PtCl ₄ :HSA (mg:mg)	0.133	0.027	0.036
Pt content of solid phase (µg/mg)	3.29	0.64	0.55
Mean RAST count for eight cord sera ± s.d. (ct/min)			
(total IgE all < 8 iu/ml)	2505 ± 18.9	1594 ± 91.3	2114 ± 94.8
IgE (iu/ml)	(a)	(b)	(c)
9000	1.54	1.72	1.52
6400	1.39	1.25	1.01
5000	1.71	1.45	1.31
4600	1.53	1.44	1.05
4111	1.27	1.42	0.96
2320	1.16	1.17	1.02
1000	1.31	1.20	0.98
603	1.36	1.36	1.02
377	1.15	1.21	1.09
235	1.08	1.13	0.80

(a), (b) = Sepharose-HSA Pt; (c) = Sepharose HSA-Pt.

Total IgE: RAST values and effects of serum dilution

Fig. 5 shows that at high levels of total IgE for the control pooled serum from four patients with allergic bronchopulmonary aspergillosis, RAST values are obtained comparable with those for sensitized workers with low levels of total IgE. When the sera of non-exposed subjects were diluted to levels of total IgE comparable to those of the sensitized workers, the latter gave significantly higher RAST values. Thus unequivocally higher RAST values were obtained for the sensitized workers as compared with the control serum at total IgE levels of 500 iu/ml or less.

Total IgE values

Fig. 6 shows that thirteen of the twenty-six skin test-positive group had raised serum total IgE and thirteen were within the 'normal' range established by our group of fifty-four healthy subjects. The nine RAST-negative/skin test-positive sera (Fig. 4) had between 20–206 iu/ml, within the 'normal' range. The geometric mean for the group was 54.4 iu/ml. The geometric mean for the RAST-positive/skin test-positive group was 457.4 iu/ml, with a range 107 to 1480 iu/ml. In the skin test-negative group only two of the twenty-one sera had total IgE values above the 'normal' range.

Serial tests

In the cases of four subjects serial serum samples taken over periods of 3 or 4 years were available. One subject followed for 3 years after cessation of exposure showed falls of prick test sensitivity from 10^{-9} to 10^{-3} g/ml; of total IgE from 1400 to 291 iu/ml and of RAST values from 2706 to 215 ct/min. In a second subject, there was a fall of total IgE from 212 to 25 iu/ml and of RAST value from 274 to zero, but no change in skin test sensitivity. In two further subjects who were still at work, but in 'clean areas', there were small falls in total IgE but not in skin test sensitivity or RAST count.

RAST with methionine and human gamma globulin conjugates with ammonium tetrachloroplatinate (II)

Of the twenty-three skin test-positive subjects, six were positive with the Sepharose

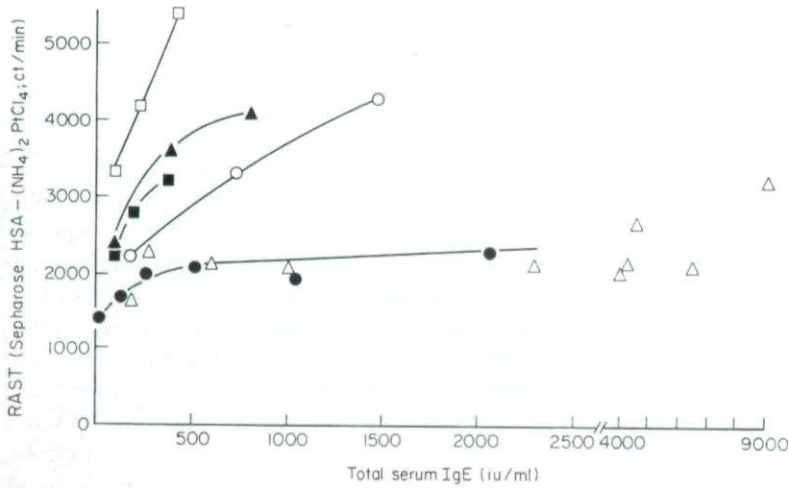


Fig. 5. RAST: total IgE of sensitized and non-exposed subjects. (□, ○, ▲, ■) Sera from sensitized subjects; (●) pool of four ABPA sera, starting from 1/2 dilution; (Δ) individual ten ABPA (total IgE).

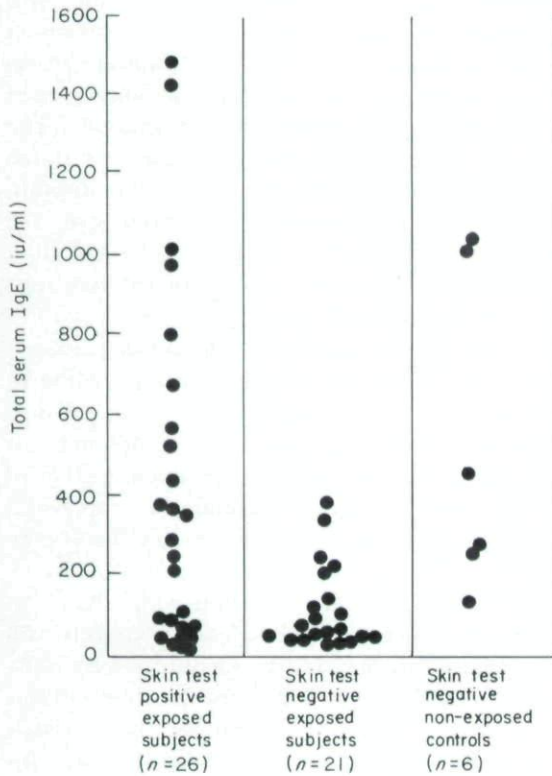


Fig. 6. Total serum IgE levels in platinum refinery workers.

methionine-Pt conjugate and eight with the Sepharose gamma-globulin-Pt conjugate. In both cases all the positives had been found positive with the Sepharose HSA-Pt conjugate. No positives were found in the skin test-negative group with either preparation.

Discussion

The clinical manifestations of platinum sensitivity have been regarded on three criteria as allergic rather than toxic or irritant (Pepys, 1977): namely, a period of exposure without symptoms; only a proportion of exposed subjects become sensitized, though this can be high because of the highly potent allergenicity of the platinum salts as shown by positive skin and/or provocation tests; and the affected subjects show sensitivity to truly minute amounts and far less than would be encountered in the ordinary way at work. The positive type I passive transfer tests in man and the monkey (Freedman & Krupey, 1968; Pepys *et al.*, 1979) and the demonstration of specific IgE antibody by the RAST in the skin prick test-positive and symptomatic workers confirms the immunological nature of the sensitivity to the inorganic platinum salts.

Antibodies to other low molecular weight chemical agents have been shown for organic chemicals such as phthalic acid anhydride—one case (Maccia *et al.*, 1976), trimellitic anhydride—3 cases (Zeiss *et al.*, 1977), tolyl- (mono) isocyanate (Karol, Ioset & Alarie, 1978) and to unconjugated azo and anthraquinone cotton dyes (Alanko *et al.*, 1978).

Reaction of the hexachloroplatinate (IV) and tetrachloroplatinite (II) salts with protein in solution, probably through sulphhydryl and methionine groups, results in cross-linking and formation of both soluble and insoluble protein: platinum aggregates (Cromwell *et al.*, in preparation). Masking of a large proportion of the platinum species within the protein matrix is possible, rendering them inaccessible to antibody. The combination of the platinum salt to the Sepharose protein conjugate was tested on the assumption that this would minimize protein aggregation by the platinum salt, thus providing better possibilities for distinguishing antibody reactions with the specific Pt hapten. The RAST results with this material showed good correlation with prick test positivity. Total IgE values were raised in one-half of the skin test-positive workers as against one-tenth of the negative subjects.

RAST tests on cord blood sera showed that the higher the amount of platinum salt combined with the HSA the higher the ct/min showing the capacity of the Pt salts to react non-specifically with IgE in the serum. Non-specific uptake of IgE was also found to be greater with the preparations in which the protein and platinum salt were combined first and then linked to the Sepharose. Non-specific uptake either of IgE or of the radiolabelled anti-IgE itself, or of both, by the platinum salt conjugates clearly reduces the sensitivity of the RAST assay, making interpretation difficult even with relevant controls.

The dose-response curves in the RAST show a fall in the counts with dilution of the sera, particularly for the control series with high total IgE values. In subjects with low total IgE and low levels of IgE antibody this may not be identified specifically. This might account for some of the nine RAST-negative/skin test-positive subjects whose total IgE values were within the 'normal' range. Furthermore, the skin tests in most of this group of subjects were made some time before blood was taken for the serological tests. Falls in both skin test, RAST positivity and levels of total IgE

have been observed in serial tests, after cessation of exposure or after transfer to 'clean areas' in the refinery.

The positive prick tests in the nine RAST-negative subjects could also have been due to other antibodies, such as heat-stable short-term sensitivity antibody, probably STS IgG. The presence of such an antibody in the sera of sensitive subjects who also had IgE antibody has been shown by passive transfer tests in man with the platinum salts (Pepys *et al.*, 1979).

The demonstration of specific IgE antibodies to the Pt salts and other well-defined chemical agents is relevant to the problems of small molecular chemical haptens in general and to drug allergy in particular, where serological tests have so far been of limited value because of the difficulties of demonstrating antibodies. It may be that the emphasis on carrier proteins, required for sensitization to haptens, is not entirely appropriate when it comes to elicitation of reactions and to serological tests. Both the Pt salts and the cotton dyes give type I skin test reactions in tests with unconjugated material. The linking of the hapten to a carrier *in vivo* probably occurs, but perhaps in a way different from that from *in vitro* conjugation. In the latter, it may be that the small molecular chemical substances are rendered less available or unavailable for reaction with the hapten-specific antibodies. In the Pt-protein conjugates with HSA there were about thirty parts of Pt per 10^6 protein, and with their affinity for sulphhydryl bonds these were probably lying within the protein molecule and hindered sterically from access to or by antibody.

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