Occupational asthma from nickel sensitivity: I Human serum albumin in the antigenic determinant

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ABSTRACT Occupational asthma from nickel sensitivity was confirmed in a male worker (SB) by allergy skin tests and inhalational challenge. In an ammonium sulphate coprecipitation test, a nickel-colour precipitate was selectively precipitated from SB plasma indicating antibody with nickel related specificity. Preincubation of nickel with human serum albumin (HSA) increased the specificity of the coprecipitation test. Blocking experiments with nickel and copper(II) salts effectively inhibited the binding of nickel to antibody of SB plasma but did not affect control tests or that for antibodies to an unrelated antigen, ampicillin. Co²⁺ slightly inhibited the binding of nickel, while Zn²⁺ and Mn²⁺ failed to inhibit. This deactivation pattern corresponds to the known sequence of binding of these metals to the primary Copper binding site of HSA. It is concluded that the antigenic determinant depends on the nickel binding site of HSA at this specific copper/nickel plasma transport site.

Occupational asthma induced by low molecular weight substances is an increasing problem. The general features of the pathogenesis of asthma on an immunological basis due to IgE antibody are reasonably well understood. By contrast, asthma induced by reactive chemicals such as toluene diisocyanate (TDI) is poorly understood and most cases are unassociated with a detectable immune response. Most of these chemically reactive inducers of asthma are new to the human experience and, at least in part, account for the apparent increase in frequency of the disease. One possible explanation for the failure to account for the disease by the demonstration of antibodies is a use of inappropriate antigen preparations.

McConnell et al were the first to document a case of asthma associated with the inhalation of nickel salts. Earlier, an incident of nickel related asthma due to exposure to nickel carbonyl had occurred in a patient with Löffler's syndrome. More recently, two additional case studies have appeared, including our own. Generally, the patients exhibited dermatitis and a positive allergy skin test to nickel salts and responded to an inhalation challenge with nickel sulphate. McConnell et al concluded from skin and haemagglutination tests that circulating antibody to nickel was implicated. Our findings appear to confirm this conclusion, as the patient's serum reacted in a Ni-HSA radioallergosorbt test for antibodies of the IgE class. In addition, in an ammonium sulphate coprecipitation (Farr) test with nickel, more label was precipitated from his plasma than control plasma specimens, further indicating the presence of antibody with Ni specificities. Coupling of Ni²⁺ with human serum albumin (HSA) appears to be required for antigenic activity. The correleative roles of Ni²⁺ and HSA in the antigenic determinant are explored in detail in this and a companion paper. Although occupational asthma due to antibodies with nickel related specificity is uncommon, the immunological principles evolved from our work should enhance our understanding of more common antibody dependent reactivity to metals, particularly to platinum.

Binding of Ni²⁺ to HSA

Binding of Ni²⁺, including that in serum, to HSA, has been shown at physiological pH values. Autoradiography experiments in basic solution (pH 8.6) have shown that Ni²⁺ occupies the primary copper binding site of HSA. Judging from spectrophotometric data for the formation of the Ni-HSA complex and the magnitude (6 × 10⁹M⁻¹, but see ref 14) of the corresponding association constant at pH 7.5, this mode of complexing is
also important at pH 7.4.11,15,16

The primary copper binding site of HSA consists of a square-planar chelate ring formed by the N-terminus α-amino nitrogen, the first two peptide nitrogens, and the 3-nitrogen of the imidazole ring of residue 3.15,17 This site is common to several albumins including bovine, human, and rat.11,18 Metal ion competition studies have shown that a threefold excess of Ni2+ suppressed the Cu2+ occupancy at this site to 5% (for comparison, the Cu2+ association constant is 9 × 10^9 M^-1, but see ref 14).13,15,19 By contrast, the same excess of Co2+ replaced only 50% of the Cu2+. Interestingly, Zn2+ did not compete with the Cu2+ and, like Mn2+, is suspected to have a different mode of attachment.15,20-22 Because of these divergent affinities for HSA, metal ion competition studies were considered potentially applicable to the characterisation of the Ni2+/HSA/antibody interactions.

Materials and methods

COLLECTION OF SAMPLES
Serum and ACD plasma were obtained from a worker (SB) in whom occupational asthma from the inhalation of nickel sulphate had been proved by inhalation challenge.5 Control serum specimens were obtained from seven laboratory workers with no occupational exposure to Ni. Two ACD plasma specimens were obtained from the local Red Cross blood transfusion service; one was group A, Rh negative and the other was group B, Rh negative.

EFFECT ON HSA OF THE ANTIBODY COPRECIPITATION TEST
The ammonium sulphate 63Ni/antibody coprecipitation (Farr) test included routine procedures.7,23 The effect of preincubating the labelled Ni2+ with HSA was examined in the following manner. Twenty microlitres of HSA (1 mg/ml distilled water) or distilled water were incubated with 100 μl of 63Ni, diluted 1/10 000 with borate-saline buffer (pH 8.3, 0.10 M H3BO3, 0.025 M Borax, 0.075 M NaCl) from prepared stock (63NiCl2, New England Nuclear, 7 × 10^-5 M Ni, 2mCi in 0.2 ml of 0.5 M HCl; and diluted to 5.0 ml with distilled water). The 63Ni-HSA or control mixtures were incubated at room temperature for two hours and then 0-1 ml test serum was added. After overnight incubation at 4°C, 0.78 ml of the borate-saline and 1.0 ml of saturated ammonium sulphate were pipetted in. After centrifugation, the precipitate was washed with 50% (NH4)2SO4, in borate-saline, and the radioactivity was then measured by liquid scintillation spectrometry after allowing the 63Ni to stabilise in the counting cocktail overnight.23

METAL ION COMPETITION STUDIES
Blocking by metal ions of the ammonium sulphate 63Ni/antibody coprecipitation test was studied using the following procedure. Twenty microlitres of the appropriate metal salt solution (10^-4 to 10^-2 M in distilled water, or in the case of Cr^3+ in 10^-2 M HNO3) were incubated overnight at ambient temperature with 100 μl of plasma. Subsequently, 120 μl of a 63Ni/HSA mixture (10 ml of 63Ni diluted in borate-saline buffer at pH 8.3 incubated for two hours with 2 ml of 1 mg/ml HSA) was added. After overnight incubation at 4°C, the (NH4)2SO4 coprecipitation test was carried out as before.

Results
Preincubation of 63Ni with HSA before addition to SB serum, in the ammonium sulphate coprecipitation test, had little effect on the precipitation of 63Ni (fig 1). This contrasts with the major reduction in the precipitation of 63Ni from control sera.

Inhibition by nickel, copper, and cobalt salts of 63Ni coprecipitation with SB plasma is illustrated in fig 2a. On a molar basis, NiSO4 had the most potent blocking effect followed by CuSO4 and then Co(NO3)2. In separate experiments NiCl2 and Ni(NO3)2 were shown to be comparable with NiSO4. From experiment to experiment, the absolute counts for SB and control samples varied slightly due to

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**Fig 1** Influence of HSA on coprecipitation of 63Ni. Serum samples were incubated with preformed 63Ni-HSA before ammonium sulphate coprecipitation test. Error bars shown correspond to relative standard error for two to six replicates calculated from variances of component terms.
settling of the protein precipitate in the liquid scintillation cocktail mixture. This process did not alter the shape of the inhibition or control curves.

The NiSO_4, CuSO_4, and Co(NO_3)_2 had no effect on the precipitation of 63Ni in tests with the two control plasma specimens. Moreover, these salts did not affect coprecipitation of an immunologically unrelated antigen with its corresponding antibodies. Thus, for the same concentration range, these salts produced no blocking of the antibody related coprecipitation of a labelled ampicillin-HSA reagent in the ammonium sulphate coprecipitation test.

Cr(NO_3)_3, ZnSO_4, and MnSO_4 had no effect on the precipitation of 63Ni from SB antibody containing plasma or from control plasma specimens (fig 2b).

**Discussion**

**EFFECT OF HSA ON THE ANTIBODY COPRECIPITATION TEST**

The observed reduction (fig 1) in binding of labelled Ni to normal human sera as a consequence of prior addition of HSA denotes reduced availability of the 63Ni^2+ for non-specific protein binding in the coprecipitation test. This decrease would also apply to SB serum. Thus the relatively small change in counts actually observed with SB serum is taken to indicate a concomitant equalising increase in specific antibody binding by this antibody containing serum. The quantity of HSA added to the labelled Ni before incubation with serum corresponded to 1/200 the quantity of HSA present in the test serum aliquot. Consequently, it may be concluded that the preformation of the 63Ni^2+/HSA complex, rather than simply an HSA concentration increase was responsible for the enhancement of interaction of 63Ni with antibody. Data on the pH dependence of the coprecipitation test for SB plasma reported in part II confirm this; they parallel that for the formation of the primary Ni^2+/HSA complex.8 On a more practical note, the use of HSA preincubation improved the specificity of the coprecipitation antibody test and was subsequently incorporated into the procedure.

**METAL ION COMPETITION STUDIES**

Excellent agreement was observed for SB plasma between the blocking effectiveness by metal ions and their known affinity for the primary copper binding site of HSA. There was blocking by Ni^2+, Cu^2+, and Co^2+ but not by Zn^2+, Mn^2+, and Cr^3+. Furthermore, there was no anion (Cl^−, NO^−_3, SO^2−_4) dependence. Metal ion or anion inhibitory effects were not observed for control samples. Ni^2+ and Cu^2+ had no effect on labelled antigen binding in an ammonium sulphate coprecipitation test of serum with antiampicillin antibodies performed with labelled HSA-ampicillin reagent (see part II).8 These observations show that the antigenic determinant recognised by antibody in SB plasma required the occupation of the primary copper binding site of HSA by Ni^2+. When Ni^2+ was displaced but remained in the reaction mixture binding to antibody diminished.

**Ni/HSA COMPLEX IN THE ANTIGENIC DETERMINANT**

The patient's sensitivity therefore seems to depend on the interaction of Ni^2+ with a native binding site on HSA and would appear to result from a unique host susceptibility. The molecular configuration of the Ni-HSA complex would presumably be "normal" but present in greater concentration in SB as a
result of the high occupational exposure to soluble nickel salts. Plasma Ni²⁺ concentrations are known to be enhanced in workers exposed to nickel in electroplating processes.¹⁰ ¹¹ The greater inhibitory effect of non-labelled Ni²⁺ compared with Cu²⁺ exhibited in fig 2a is surprising since the Cu²⁺/HSA interaction is stronger by at least a factor of 15 (see association constants quoted above). This observation implies that the strength of the Ni²⁺, HSA/antibody interaction depends on a special structural feature of the interaction of Ni²⁺ with HSA.

**IMPLICATIONS**

HSA was used as the binding protein in these experiments because we have knowledge of the specific binding site for Cu²⁺ and Ni²⁺. In individuals reacting to other low molecular weight substances alternative host components might be more relevant, although in the case of the metal sensitiser platinum, the reactive RAST antigen was Pt reacted with HSA.¹⁸ Recently, a specificity of antibody for trimellitic anhydride (TMA)-modified secretory IgA has been postulated for individuals with TMA induced occupational asthma. Specificity for carrier HSA has also recently been reported for antibodies to TDI and TMA.¹⁹ ²⁰ This does not constitute interaction dependent on a normal physiological binding site on the protein comparable to the Cu²⁺ binding site on HSA. Nevertheless, TDI or TMA induced modification or denaturation could occur in vivo and represent the molecular basis for the stimulation of antibody and the resulting sensitisation. Thus alterations of host components may be on the basis of a normal binding site provided by the host or a denaturation produced by highly chemically reactive inhaled molecules such as TDI and TMA.

Regardless, the recognition that the novel antigenic determinants yielded by these alterations can potentially be a basis of sensitisation may well usher in new approaches to the unravelling of the pathogenesis of reactions to environmental substances including those leading to occupational asthma. We are currently investigating the possibility that the requirement of the Ni/HSA complex for immunological reactivity could also apply to cellular immune responses operative in contact dermatitis and reactions to metal prostheses. The leukocyte migration test indicates that this may be so.²⁸ Thus comparable mechanisms pertaining to antigenic specificity may well be involved in both humoral and cellular responses to nickel, and by analogy, presumably also to cobalt, platinum, and other metals. Clarification of these issues might be expected to improve the laboratory tests available to identify sensitised individuals.

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**References**

 Antibody with Ni\textsuperscript{2+} specificity reacts with Ni\textsuperscript{2+}/HSA complex


