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Occupational asthma from nickel sensitivity: II Factors influencing the interaction of Ni^{2+}, HSA, and serum antibodies with nickel related specificity

E NIEBOER, S L EVANS, AND J DOLOVICH

From the Departments of Biochemistry and Pediatrics, Faculty of Health Sciences, McMaster University, Hamilton, Ontario, Canada L8N 3Z5

ABSTRACT Serum from an individual with occupational asthma from nickel sulphate inhalation was shown by ammonium sulphate (Farr) and anti-IgG coprecipitation tests to contain antibodies which bind $^{63}Ni^{2+}$. This binding was absent from 30 control sera. Ligand competition studies with serum and plasma from the propositus and controls are described, and include serial additions of HSA, L-histidine, EDTA, and the tripeptide gly-gly-L-his. The results, like those from metal ion blocking experiments, clearly showed that the formation of the reactive antigenic determinant depends on the selective binding of Ni^{2+} at the native Cu^{2+}/Ni^{2+} transport site of HSA. Corroborating evidence is derived from the similar dependence on pH of the Ni^{2+}-HSA formation and the ammonium sulphate $^{63}Ni^{2+}$ coprecipitation reactions.

In part I we applied principles of inorganic biochemistry in a study of the antigenic determinant of antibody with Ni^{2+}-related specificity.[1] The serum of worker SB, who had occupational asthma from nickel sulphate inhalation, had previously been shown to react in a Ni-HSA radioallergosorbant test for antibodies of the IgE class.[2] The presence of antibody with nickel related specificity was confirmed by the selective precipitation of $^{63}Ni^{2+}$ in an ammonium sulphate induced coprecipitation reaction.[1] Preincubation of the labelled nickel with human serum albumin (HSA) increased the discrimination of this coprecipitation test. Furthermore, metal ion blocking experiments indicated strongly that the antigenic determinant depended on the combination of Ni^{2+} with HSA at the specific copper-nickel transport site located at its N-terminus. In the present paper new evidence is presented for the presence of antibody with Ni^{2+} recognition in SB's serum (plasma), and the specificity of the Ni^{2+}-HSA requirement is documented and explored further.

To augment the characterisation of the sensitising role and a consequent requirement of HSA in detection of antibody by the ammonium sulphate coprecipitation test, an HSA dose response curve is reported for the allergic individual (SB) and for non-allergic control plasma samples. A comparison is also made of the pH dependence of formation of the Ni^{2+}-HSA complex with that of the coprecipitation reaction. Moreover, ligand competition is explored as a probe since some ligands are known to compete effectively at physiological pH values with HSA for Ni^{2+}. Sarkar and co-workers have shown that L-histidine and tripeptides mimicking the primary binding mode of HSA form complexes of comparable stabilities to HSA with Ni^{2+} and Cu^{2+}.[3–7] As might be expected from its high affinity for the nickel ion,[8] EDTA has been shown to remove 90% of Ni^{2+} from HSA when both reagents were at comparable concentrations.[9] Ligand inhibition of $^{63}Ni^{2+}$ coprecipitation might therefore be expected for L-histidine, gly-gly-L-his, and EDTA, and should provide another avenue for testing the hypothesis concerning the central role of the Ni^{2+}-HSA complex.

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Materials and methods

COLLECTION OF SAMPLES

Serum and ACD plasma were obtained from SB, a worker in an electroplating plant, with known occupational asthma from $NiSO_4$ inhalation, positive $NiSO_4$ allergy skin tests, and antibodies with nickel related specificity shown by a Ni/HSA-sepharose radioallergosorbant test (RAST) and ammonium sulphate coprecipitation test (Farr).[1 2] Two control

ACD plasma specimens were obtained from the blood bank of the Canadian Red Cross blood transfusion service. The clinical immunology laboratory of the McMaster University Medical Centre provided samples of 30 control sera from specimens submitted for routine diagnostic tests.

REAGENTS

The labelled reagent, $^{63}NiCl_2$ (7×10^{-4} M Ni, 2 mCi in 0·2 ml of 0·5 M HCl) was obtained from New England Nuclear Corporation. For use, it was diluted to 5·0 ml with distilled water. Ammonium sulphate was obtained from BDH Chemicals, Toronto. A saturated solution was shown to contain less than 1 ng/ml of nickel by electrothermal atomic absorption spectrometry.

Additional sources of reagents were as follows: $NiSO_4$, $Co(NO_3)_2$ and $ZnSO_4$, BDH; $CuSO_4$, $MnSO_4$, and EDTA, Baker Chemical Co; $Cr(NO_3)_3$, Allied Chemical, NY; $NiCl_2$, Fisher Scientific Co; lipid-free HSA, lot No 110F-9350, and L-histidine, Sigma Chemical Co; gly-gly-L-his, Peninsula Laboratories, San Carlos, Ca. All metal salts were of reagent grade.

Normal rabbit serum was obtained from rabbits housed in the animal quarters of the McMaster Health Sciences Centre. Rabbit antiserum to human serum albumin was prepared by the immunisation of rabbits with HSA incorporated into complete Freund's adjuvant (Difco Corporation).

A labelled ampicillin-HSA reagent was prepared as follows.[10] One milligram HSA (Sigma) in 1 ml distilled H_2O was reacted with 2·0 mCi ^{125}INa in the presence of 0·25 mg chloramine T, then the reaction was stopped by the addition of 0·5 mg sodium metabisulphite and KI (1 mg). The iodinated HSA was passed through a Sephadex G-50 superfine column with borate buffer (pH 8·3) and 0·2 ml aliquots were collected. The fractions containing the highest amounts of ^{125}I were pooled ($\simeq$2·2 ml) and dialysed against borate buffer. Ampicillin sodium (Ayerst; 6 g) was dissolved in 60 ml distilled H_2O adjusted to pH 10. About 1 ml of the ^{125}I-HSA was transferred to a dialysis bag, which was left to dialyse overnight at 4°C in the ampicillin solution. The ^{125}-HSA-ampicillin was then washed six times with 500-600 ml PBS, and subsequently the contents of the dialysis bag were passed through superfine Sephadex G-50, the column was eluted with buffer, and the fractions with the highest counts were pooled.

^{63}Ni COPRECIPITATION (FARR)[11] TEST

Initially, $^{63}NiCl_2$ was diluted 10 000-fold with borate saline buffer (pH 8·3, 0·10 M H_3BO_3, 0·025 M Borax, 0·075 M NaCl). Then 100 μl labelled nickel was added to 100 μl serum and this was incubated overnight at 4°C after which 0·8 ml borate buffer and 1·0 ml saturated ammonium sulphate were pipetted in. After centrifugation, the precipitate was washed with 50% ammonium sulphate in borate saline buffer and the radioactivity was measured by liquid scintillation spectrometry.[12] Measurements under various conditions showed that chemiluminescence could be avoided by allowing the ^{63}Ni to stabilise in the counting cocktail overnight before counting. All results are mean values of three replicate tests.

IgG COPRECIPITATION TEST

An IgG coprecipitation test was accomplished with antiserum to human IgG obtained from the central resource for immunologic reagents of McMaster University. The test was performed with SB serum and two control sera in a manner identical to the ammonium sulphate coprecipitation test until the end of the overnight incubation at which time 0·8 ml of the goat antihuman IgG was added to the incubation mixtures. The volume added had been determined by prior measurements of the volume of the particular antiserum required to produce maximal precipitation of protein from 0·1 ml normal human serum. After an additional overnight incubation, the insoluble precipitate was washed three times with borate-saline buffer and the ^{63}Ni counts were then measured by the same method as in the Farr test.

LIGAND COMPETITION STUDIES

To examine the effect of lipid-free HSA, 20 μl freshly prepared solutions in concentrations of $1{\cdot}5 \times 10^{-4}$ M (10 mg/ml), $0{\cdot}50 \times 10^{-4}$ M, $1{\cdot}5 \times 10^{-5}$ M, $0{\cdot}50 \times 10^{-5}$ M, and $1{\cdot}5 \times 10^{-6}$ M in distilled H_2O or H_2O control were added to 100 μl $^{63}Ni^{2+}$ in borate saline buffer (pH 8·3) and incubated for two hours. The mixture was then added to 100 μl plasma for overnight incubation. The effect of EDTA was examined by adding 10 μl EDTA (10^{-1} M, 3×10^{-2} M, 10^{-2} M, and 10^{-3} M solutions in borate buffer) or borate buffer control to 100 μl serum and incubating for 2 h, followed by the addition of 100 μl labelled Ni^{2+} in buffer and overnight incubation. To determine the effect of L-histidine, 20 μl L-histidine (10^{-1} M, 3×10^{-2} M, 10^{-2} M, or 10^{-3} M in borate buffer, pH 9·3) or buffer control alone was incubated with 100 μl $^{63}Ni^{2+}$ in borate saline for 2 h, followed by the usual addition to 100 μl serum for overnight incubation at 4°C. For studies with the tripeptide gly-gly-L-his, 100 μl of $^{63}Ni^{2+}$ in borate buffer was incubated for 2 h at ambient temperature with 20 μl of freshly prepared HSA ($1{\cdot}5 \times 10^{-5}$ M) or distilled water and 20 μl of freshly prepared pep-

tide ($3\cdot3 \times 10^{-3}$ M, $3\cdot3 \times 10^{-4}$ M, or $3\cdot3 \times 10^{-5}$ M) or distilled water. Subsequently 100 μl of plasma was added followed by overnight incubation at 4°C before the coprecipitation reaction.

METAL ION STUDIES

The effect of nickel, copper, cobalt, manganese, and chromium salts was examined in tests performed with SB and control plasma specimens by the addition of the appropriate metal ion in 20 μl amounts to 100 μl of plasma followed by overnight incubation at room temperature. There was then the addition of a mixture of 100 μl ^{63}Ni and 20 μl of HSA ($1\cdot5 \times 10^{-5}$ M), followed by another overnight incubation at 4°C, and the ammonium sulphate coprecipitation reaction. The molar concentration of non-labelled salt required to produce a 50% inhibition (IC_{50}) of ^{63}Ni coprecipitation for SB plasma was derived from a semilog plot of the observed counts corrected for background. The latter was taken as the counts for non-antibody control sera tested in the same experiment (see for example, fig 2A, part I).[1]

The effect of nickel and copper sulphate on ampicillin coprecipitation from an antibody containing serum was examined in the manner just described except for two modifications: (1) the ampicillin-^{125}I-HSA was the labelled reagent rather than $^{63}Ni^{2+}$/HSA and (2) radioactivity measurements were by an automatic gamma counter.

pH DEPENDENCE OF COMPLEX FORMATION AND COPRECIPITATION

Difference spectra were recorded (Varian DMS 90 UV/visible spectrophotometer) in the wavelength range 350–750 nm for solutions prepared in 0·15 M KCl containing 10^{-3} M HSA + 10^{-3} M Ni^{2+} (the sample) and 10^{-3} M HSA (the reference sample). Solutions of each type were prepared with pH values between six and nine by mixing appropriate aliquots of parent solutions of pH 6·0 and 9·0. Samples were centrifuged before the spectrophotometric measurements. pH adjustments of the parent solutions were made with 1 M KOH or 1 M HCl.

Phosphate (0·2 M, pH 6–8) and borate buffers (0·1 M, 0·45% NaCl, pH 7·6–9·10) were used in the examination of the pH dependence of the ammonium sulphate coprecipitation test. $^{63}Ni^{2+}$/HSA in buffer (120 μl) and plasma (100 μl) were incubated overnight at 4°C. Subsequently, 780 μl of buffer and 1·0 ml of saturated $(NH_4)_2SO_4$ were added to induce precipitation. Precipitates were washed with 50% $(NH)_2SO_4$ prepared in the appropriate buffer.

Results

Table 1 illustrates the comparison of SB serum with control sera in the ammonium sulphate test. In agreement with earlier results[1], counts for SB serum were about threefold higher than counts for control serum specimens A and B. When SB serum was serially diluted in normal human serum, counts at the 1/4 dilution were above control serum but at 1/8 dilution, SB counts approximated counts with normal human serum. The relation between SB serum and control sera was similar to the relation between SB plasma and control plasma specimens, except that slightly lower counts with plasma reflected the 30% dilution factor for ACD plasma. Thirty control sera all had similar results with a mean ± 1 SD counts of 57 ± 9. There were no outlying values comparable with SB. Coprecipitation by the anti-IgG method was also positive for SB serum with 679 precipitated counts compared with counts for two control serum specimens of 262 and 315 respectively (table 1).

IC_{50} values for various metals in the $^{63}Ni^{2+}$ ammonium sulphate coprecipitation test are compiled in table 2. The order, in decreasing capacity to inhibit, is Ni^{2+}, Cu^{2+}, and Co^{2}; Zn^{2+}, Mn^{2+}, and Cr^{3+} did not inhibit. There was no significant anion effect, or effect of any of the salts on counts obtained with control plasma specimens. In addition, nickel sulphate and copper sulphate (fig 1) had no effect on the ammonium sulphate coprecipitation

Table 1 *Demonstration of antibody activity by ammonium sulphate and IgG coprecipitation of $^{63}Ni^{2+}$*

Sample	*Ammonium sulphate (cpm ± SE)*	*Anti-IgG (cpm ± SE)*
SB serum	163 ± 8	679 ± 23
Control sera		
A	63 ± 5	262 ± 10
B	74 ± 3	315 ± 35
Total counts added	6036	6004

Table 2 *Metal ion inhibition of ^{63}Ni coprecipitation*

Metal salt	*Dose at 50% inhibition (IC50, moles/l)* SB plasma*	*Control plasma*
$NiCl_2$	7×10^{-7}	Nil
$Ni(NO_3)_2$	10^{-6}	Nil
$NiSO_4$	2×10^{-6}	Nil
$CuSO_4$	5×10^{-5}	Nil
$Co(NO_3)_2$	5×10^{-3}	Nil
$ZnSO_4$	Nil	Nil
$MnSO_4$	Nil	Nil
$Cr(NO_3)_3$	Nil	Nil

*These concentrations refer to the samples during the final overnight incubation (at 4°C, 0·2 ml total volume) before dilution in the Farr coprecipitation test.

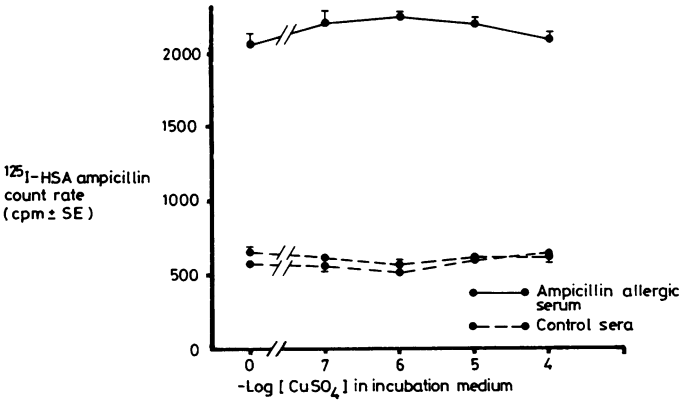

Fig 1 *Effect of Cu^{2+} on precipitation of antibodies to iodine-labelled HSA/ ampicillin complex. Non-addition is denoted by zero on concentration scale in this and subsequent figures.*

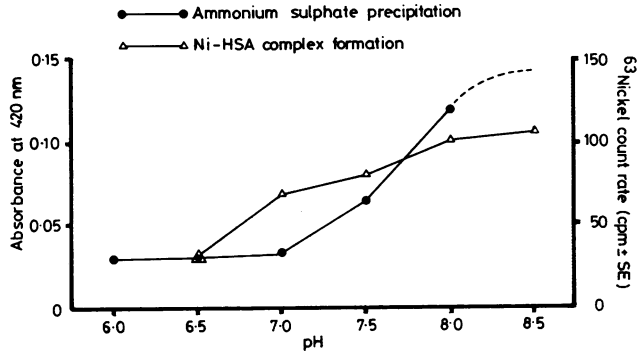

Fig 2 *Formation of Ni^{2+}-HSA complex and ammonium sulphate $^{63}Ni^{2+}$ coprecipitation test as a function of pH. Extrapolated segment for ammonium sulphate coprecipitation test was confirmed in a separate experiment.*

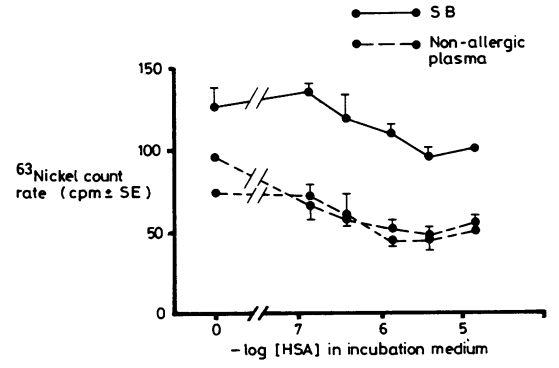

Fig 3 *Coprecipitation response curves to preincubation of $^{63}Ni^{2+}$ with HSA. Abscissa HSA concentrations refer to amount added with the ^{63}Ni label to a native level derived from plasma of $\simeq 2 \times 10^{-4}$ M.*

test performed with the labelled ampicillin reagent and human ampicillin antiserum

Difference spectra recorded for the Ni^{2+}/HSA complex confirmed those reported recently by Glennon and Sarkar.[7] In the present study spectra exhibited a single peak with λ_{max} = 420 nm and ϵ = 110 l $cm^{-1}mol^{-1}$, which is indicative of a square planar or square pyramidal geometry about the Ni^{2+} ion. Although of low absorbance as indicated by the first point in the complex formation curve in fig 2, the presence of a well defined peak at pH 6·5 in the visible difference spectrum showed that complex formation had begun; while the levelling off in absorbance at pH values > 8·0 signalled its completion. It is evident from fig 2 that Ni^{2+}-HSA complex formation and the $^{63}Ni^{2+}$ coprecipitation test exhibited a similar dependence on pH.

Preincubation of $^{63}Ni^{2+}$ with HSA in an amount which yielded a final HSA concentration of $1·5 \times 10^{-6}$ (0·1 mg/ml) has been shown in repeat experi-

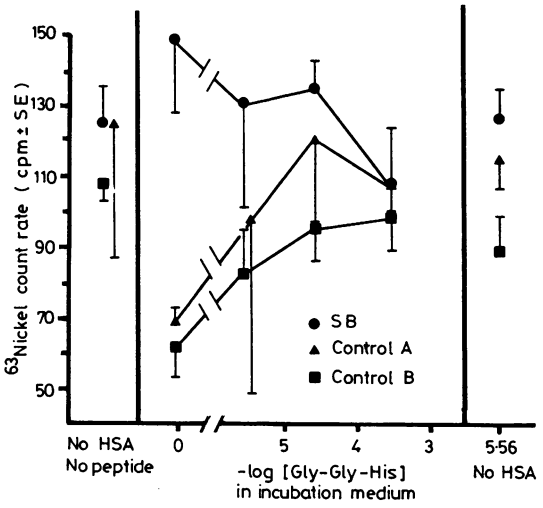

Fig 4 *Effect of tripeptide gly-gly-L-his on HSA-induced discrimination enhancement in the $^{63}Ni^{2+}$ coprecipitation test for SB plasma relative to controls.*

ments to lower the counts with control plasma specimens by 45% but with little effect on $^{63}Ni^{2+}$ coprecipitation with SB plasma.[1] A dose response plot of the effect of HSA is provided in fig 3. At the highest concentrations, increasing quantities of HSA reduced counts with both the antibody (SB) and non-antibody-containing sera. The discrimination between SB and control plasma was maximum at HSA concentrations between 10^{-6}–10^{-7} M.

The copper binding tripeptide gly-gly-L-his was tested in the presence of HSA and, in effect, reversed the effects of HSA with a reduction in coprecipitation of $^{63}Ni^{2+}$ with SB plasma and an increase in coprecipitation with control plasma specimens (fig 4). The preincubation of the peptide alone with $^{63}Ni^{2+}$ had no effect.

The influence of preincubating sera with disodium EDTA, a substance which chelates Ni^{2+}, is illustrated in fig 5. At the lowest concentration, there was an increase in $^{63}Ni^{2+}$ coprecipitation, particularly with SB plasma. At the largest concentrations of EDTA, the coprecipitation of $^{63}Ni^{2+}$ was virtually abolished. By contrast (fig 5), disodium EDTA did not affect the coprecipitation of the protein antigen (labelled HSA) with the corresponding antibody (rabbit anti-HSA).

L-histidine when preincubated with $^{63}Ni^{2+}$ at concentrations in molar excess of the added label (total Ni^{2+}, 3×10^{-8} M) produced results resembling those of EDTA (fig 6). At the highest concentrations, this amino acid reduced the $^{63}Ni^{2+}$ coprecipitation with both SB serum and control sera but did not affect the ammonium sulphate coprecipitation test involving the organic antigen, ampicillin, in reaction with antibody to ampicillin.

The concentrations indicated on the abscissae of figs 1 and 3–6 refer to the sample mixtures incubated overnight at 4°C (usually 0·2 ml total volume), and non-addition is denoted by zero on the concentration scale.

Discussion

COPRECIPITATION OF $^{63}Ni^{2+}$ WITH IgG

The increased coprecipitation of labelled nickel from SB serum in the ammonium sulphate coprecipitation concurs with antibody binding. The evidence presented in table 1 that a higher level of coprecipitation also occurred from SB serum in the

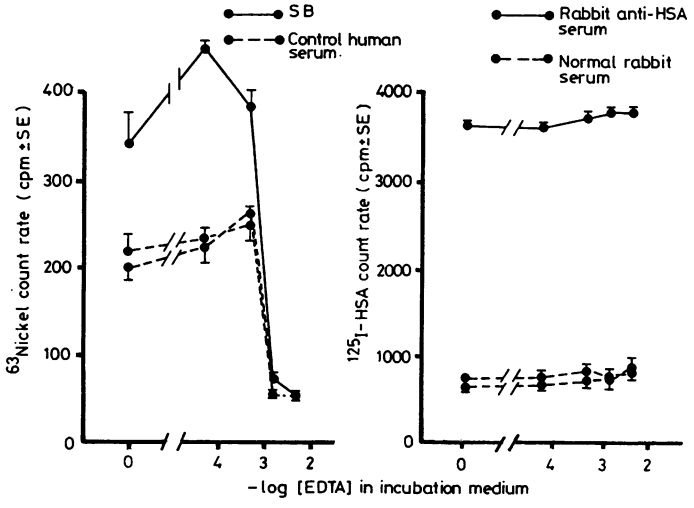

Fig 5 *Effect of preincubating sera with the chelating agent EDTA before coprecipitation of $^{63}Ni^{2+}$ or protein antigen. In the latter case 100 µl of ^{125}I-HSA rather than 100 µl of $^{63}Ni^{2+}$ were added as the label after the EDTA/serum preincubation step (see text).*

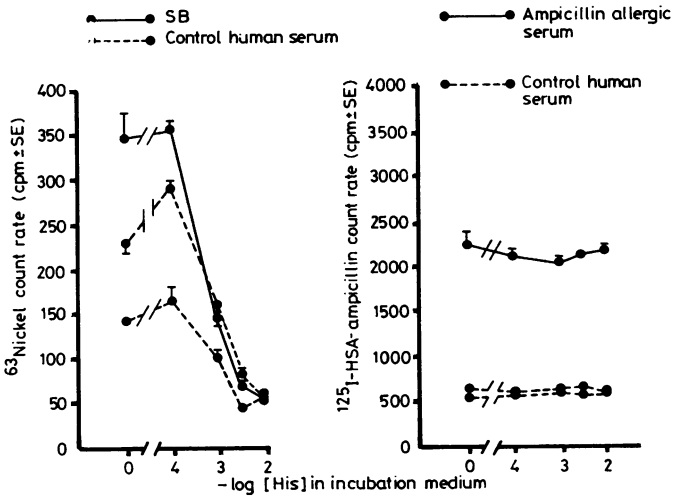

Fig 6 *Effect of preincubating $^{63}Ni^{2+}$ with the amino acid L-histidine before coprecipitation of $^{63}Ni^{2+}$ or ^{125}I-HSA-ampicillin. In the latter case 100µl of ^{125}I-HSA-ampicillin rather than 100 µl of $^{63}Ni^{2+}$ were incubated with 20 µl of the L-histidine solution before addition of serum (see text).*

anti-IgG coprecipitation test is stronger evidence for the presence of antibody to nickel. This supports our previous conclusion based upon a $NiSO_4$-HSA-sepharose test for IgE antibodies, that SB serum contains antibody with nickel related specificity.[2]

pH DEPENDENCE OF COMPLEX FORMATION AND COPRECIPITATION

The good agreement between the two curves in fig 2 provides further evidence that the binding of Ni^{2+} to the primary Cu^{2+}/Ni^{2+} transport site of HSA is required for its recognition by antibody. As documented by Glennon and Sarkar,[7] the absorption peak at 420 nm is characteristic of the involvement of two deprotonated amide nitrogen atoms of the N-terminal sequence asp-ala-his of HSA, as well as the α-amino and histidine imidazole nitrogen donor atoms. Nuclear magnetic resonance data also implicate the involvement of the side chain carboxylate group of the aspartic acid residue. The Cu^{2+}-binding site appears to have a similar "pentacoordinate, pyramidal geometry."

It is evident from the ammonium sulphate coprecipitation curve in fig 2 that the coprecipitation test is most sensitive at pH $\geq$ 8.

METAL ION EFFECTS

The blocking effects observed for SB plasma or serum of non-labelled Ni^{2+}, Cu^{2+}, and Co^{2+} were not evident for control plasma specimens. Presumably this blocking is due to a competition for binding sites which must be considered to be antibody related due to the exclusive effect on SB plasma. The absence of any effect due to metal ions on labelled antigen binding in the coprecipitation of ampicillin from serum with antiampicillin antibodies (fig 1) attests to the specificity of the inhibitory effect observed for SB samples. As summarised previously,[1] Ni^{2+} and Cu^{2+} have the highest affinity for the copper transport site of HSA. Co^{2+} has a lower binding affinity, while Zn^{2+}, Mn^{2+}, and Cr^{3+} cations do not bind there at all. At least qualitatively, the potency of the inhibitory effect would appear to parallel the binding capacity of the ions at this specific site, since the IC_{50} values reported in table 2 are in the ratio of 1 (Ni^{2+}):40(Cu^{2+}):4000(Co^{2+}). Since, however, the NiHSA complex has been reported to be considerably less stable (with a formation constant log K_{NiL} = 9·6, at 6°C and μ = 0·16)[7] than the CuHSA species (log K_{CuL} = 16·2, at 6°C and μ = 0·16),[3] thermodynamic arguments might have predicted that Cu^{2+} would be more effective than non-labelled Ni^{2+} in displacing $^{63}Ni^{2+}$ in the coprecipitation test. Since this did not occur, it may be concluded that the HSA-Ni-antibody interaction is highly specific. The basis for this selectivity is not clear. Perhaps special structural features of NiHSA not present in CuHSA are responsible for the implied unusual stability of the ternary complex between Ni^{2+}, HSA, and antibody.

LIGAND COMPETITION STUDIES

An analysis of the data reported in fig 3 indicated that the discrimination between SB and control plasma samples was maximum for *added* HSA concentrations between 10^{-6}–10^{-7} M HSA. (For comparison, the total concentration of Ni^{2+} in the incubation medium due to the concomitant addition of label was 3×10^{-8} M; and the HSA derived from plasma was $\simeq 2 \times 10^{-4}$ M.) The nickel-HSA prein-

cubation step appears to assure the preformation of $^{63}Ni^{2+}$HSA, presumably making it available for the formation of the ternary HSA-$^{63}Ni^{2+}$-antibody complex on addition of SB serum or plasma. Corroborating evidence for this interpretation may be gleaned from the data in fig 4. Although the tripeptide gly-gly-L-his itself did not induce any enhancement, it abolished that initiated by HSA when present simultaneously with it in the preincubation step. In this experiment the added concentration (referred to the incubation medium) of HSA was $1{\cdot}3 \times 10^{-6}$ M and of Ni^{2+} (as the label) 3×10^{-8} M; these concentrations are roughly one half of what occurred in the preincubation medium. Since the tripeptide and HSA form complexes of similar strength (with formation constants of $\simeq$ 10 log units),[7] the $^{63}Ni^{2+}$ becomes progressively more associated with the peptide as its concentration increases to a maximum of $2{\cdot}8 \times 10^{-4}$ M (referred to the incubation medium).

Data from rabbit anti-HSA serum (fig 5) and ampicillin allergic serum (fig 6) illustrate clearly that EDTA and L-histidine do not inferfere in a fundamental manner with the ammonium sulphate antigen-antibody coprecipitation test. Consequently, the alterations induced in the $^{63}Ni^{2+}$ coprecipitation studies are correctly assigned to ligand/Ni^{2+} competition effects. It is evident from the data in figs 5 and 6 that high concentrations of EDTA and L-histidine in the incubation medium reduces the coprecipitation of $^{63}Ni^{2+}$ to background levels. All of the label would thus appear to be associated with the added ligand in both SB and control samples. The concentration required to accomplish this for L-histidine was twice as large ($2{\cdot}7 \times 10^{-3}$ M) than required for EDTA ($1{\cdot}4 \times 10^{-3}$ M). This is consistent with their relative complex stabilities (log K_{NiL}(his) = 8·6; log K_{NiL_2} (his) = 15·6; log K_{NiL} (EDTA) = 18·6).[7,8]

The relatively large increase in $^{63}Ni^{2+}$ coprecipitation for SB serum with EDTA $< 10^{-3}$ M is interpreted to result from a reduction in the availability of non-labelled Ni^{2+} present as natural background contamination. In addition to the Ni^{2+}, this ligand would be bound to other cations in the serum such as Ca^{2+}, Mg^{2+}, Zn^{2+}, and Cu^{2+}, and therefore was not present in large excess. As may be deduced from the average IC_{50} value of $\simeq 10^{-6}$ M for Ni^{2+} salts in table 2, concentrations of added non-labelled Ni^{2+} as low as 10^{-7} M had measurable depressive effects on the coprecipitation test.[1] Background levels of 10^{-7} M ($\equiv 6$ μg/l) due to random contamination are common.[13,14] Presumably a larger fraction of the $^{63}Ni^{2+}$ added subsequently is available for incorporation into the ternary HSA-Ni^{2+}-antibody complex. And finally, the noticeable enhancement in $^{63}Ni^{2+}$ counts for control sera due to low levels of L-histidine in the incubation medium ($8{\cdot}3 \times 10^{-5}$ M) suggests that this ligand does not block the availability of $^{63}Ni^{2+}$ for non-specific binding in the coprecipitation reaction. Exchange phenomena involving Cu^{2+}, L-histidine, and HSA have been documented.[3,15] Furthermore, Ni^{2+} in serum is known to be distributed and exchanged between the binary complexes Ni-HSA, Ni(his), and Ni$(his)_2$, as well as with the ternary species HSA-Ni-his.[6,7]

Concluding remarks

The increased coprecipitation of labelled nickel in the anti-IgG coprecipitation reaction provides additional and strong evidence for the presence of antibodies to nickel in patient SB with occupational asthma from nickel sensitivity. It may be concluded that the ligand competition studies, like the metal ion blocking experiments, clearly show that the formation of the reactive antigenic determinant depends on the selective binding of Ni^{2+} at the specific Cu^{2+}/Ni^{2+} transport site of HSA. Corroborating evidence is derived from the similar dependence of pH of the Ni^{2+}-HSA formation and the ammonium sulphate $^{63}Ni^{2+}$ coprecipitation reactions. Presumably the strength of the Ni-HSA-antibody interaction depends on a special structural feature of the interaction of Ni^{2+} with HSA.

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