Biochem. J. (1981) **194**, 561–568
Printed in Great Britain

The presence in serum of proteins which are immunologically cross-reactive with Tamm–Horsfall glycoprotein

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(*Received 16 April 1980/Accepted 25 June 1980*)

Affinity chromatography, with rabbit anti-(human Tamm–Horsfall glycoprotein) IgG, was applied to the isolation from normal human serum of protein, which is immunologically cross-reactive with the urinary glycoprotein. The antigen–antibody complex was dissociated with the use of sodium thiocyanate solution, a medium which fails to dissociate urinary Tamm–Horsfall glycoprotein–antigen complex. The cross-reactive serum proteins were isolated in amounts of 19–24 mg/l of serum. They have apparent molecular weights, assessed by disc-gel electrophoresis in the presence of sodium dodecyl sulphate, of 125000, 84000 and 74000 respectively, with mobilities differing from that of urinary Tamm–Horsfall glycoprotein. They have a much lower immunoreactivity towards the antibody than does the urinary glycoprotein. Tamm–Horsfall glycoprotein could not be demonstrated in normal serum by the techniques used. The implications of these findings are discussed in terms of pathology involving Tamm–Horsfall glycoprotein.

The urinary glycoprotein named after its discoverers, Tamm & Horsfall (1950, 1952), is produced by the cells lining the ascending limb of the loop of Henle excluding the maculae densae (Sikri *et al.*, 1979; Hoyer *et al.*, 1979) and, at least in some species, by those lining the distal convoluted tubule as far as the initial collecting duct (Sikri *et al.*, 1979). It has been suggested that it provides the relatively water-impermeable barrier which must be present in the ascending limb of the loop of Henle for generation of the essential hypertonicity of the renal medulla (Sikri *et al.*, 1979; Hoyer & Seiler, 1979).

There is evidence that the glycoprotein is implicated in some pathological states. Cell-mediated immunity to the glycoprotein is reported to occur in many patients with renal tubular acidosis accompanying autoimmune liver disease (Tsantoulos *et al.*, 1974), and the lymphocytes from many of these patients have been shown to be cytotoxic for a line of cultured kidney cells (Cochrane *et al.*, 1976), possibly because the latter are producing the glycoprotein or an immunologically cross-reactive substance (Bloomfield *et al.*, 1977). There are increased levels of autoantibodies to Tamm–Horsfall glycoprotein in the sera of those with upper, but not lower, urinary tract infection (Larsson *et al.*, 1978; Fasth *et al.*, 1979) and in some patients with urinary tract obstruction accompanied by infection (Marier *et al.*, 1978). An understanding of these phenomena requires recognition of the antigen(s) involved, as well as of cross-reactive substances.

The results of applying a radioimmunoassay procedure to normal human sera were interpreted as evidence for the presence of a substance, in amounts of 0.05–0.18 mg/l, which reacted similarly to the Tamm–Horsfall glycoprotein (Avis, 1977). Dawnay *et al.* (1980) reported that non-aggregated Tamm–Horsfall glycoprotein is present in normal human serum in amounts of 0.07–0.54 mg/l, although the identity of the material was not unequivocally established.

The present results show that Tamm–Horsfall glycoprotein does not occur in normal human serum, although there are proteins which are immunologically cross-reactive with it and which have been isolated in amounts of about 20 mg/l.

Materials and methods

Suppliers of materials were as follows: complete Freund's adjuvant, Difco, Detroit, MI, U.S.A.; DEAE-cellulose (Whatman DE 52), MacFarlane Robson Ltd., Thornliebank, Glasgow, Scotland, U.K.; cyanogen bromide-activated Sepharose 4B,

Abbreviations used: NaCNS, sodium thiocyanate; SDS, sodium dodecyl sulphate.

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Pharmacia (G.B.) Ltd., Hounslow, Middlesex, U.K.; sodium dodecyl sulphate (SDS) and sodium thiocyanate (NaCNS), British Drug Houses, Poole, Dorset, U.K.; $Na^{125}I$, The Radiochemical Centre, Amersham, Bucks., U.K.; human serum albumin and agarose, Sigma London Chemical Co., Ltd., Poole, Dorset, U.K.; sheep immunoglobulin G (IgG), Pentex Biochemicals, Kankahee, IL, U.S.A.; Bio-Gel A.15m, Bio-Rad Laboratories, Richmond, CA, U.S.A.

Isolation of Tamm–Horsfall glycoprotein

The glycoprotein was isolated from pooled, adult urine by procedures described earlier (Tamm & Horsfall, 1952) and freeze-dried.

Raising of antiserum

Antiserum to freeze-dried Tamm–Horsfall glycoprotein was raised in New Zealand white rabbits by multiple intradermal injections (5 × 0.1 ml), every month, of an emulsion containing equal volumes of the glycoprotein (0.5 mg/ml of water) and complete Freund's adjuvant. Blood, taken from the ear veins of the immunized rabbits after a total of at least four sets of injections, was allowed to clot and the serum was collected. It was stored in small portions at −20°C.

Isolation of IgG from antiserum and from the serum of non-immunized rabbits

IgG was obtained by chromatographing dialysed (against starting buffer) rabbit antiserum (5 ml), or serum from non-immunized rabbits, on a column (2 cm × 12 cm) of DEAE-cellulose (DE52 Whatman) at pH 6.4 in 0.0175 M-phosphate buffer (Sober & Peterson, 1958). The rate of elution was 12 ml/h and 15 ml fractions were collected. The peak fractions containing IgG were combined and dialysed against water before freeze-drying. Yields were about 27 mg from the immunized rabbit and 21 mg from the non-immunized rabbit.

Preparation of IgG–Sepharose 4B for affinity chromatography

IgG was coupled to Sepharose 4B by a modification of the procedure of Cuatrecasas (1970). Amounts of CNBr-activated Sepharose 4B (5 g) were swollen for 15 min in a sintered-glass funnel with 1 mM-HCl (200 ml/g of Sepharose 4B).

Anti-(Tamm–Horsfall glycoprotein)IgG (25 mg) was dissolved in 50 ml of a solution of 0.1 M-$NaHCO_3$ containing 0.5 M-NaCl in a plastic container. To the solution was added a 5 g amount of the swollen Sepharose 4B gel, and the mixture was allowed to react on a roller for 2 h at 21°C. The mixture was washed on a sintered glass funnel with 0.1 M-$NaHCO_3$, containing 0.5 M-NaCl, and A_{280} measurements of the collected washings indicated binding of 70–80% of the IgG.

The IgG–Sepharose 4B preparation was mixed with 1 M-ethanolamine/HCl (pH 8, 30 ml) for 2 h at 21°C on the roller. The supernatant was decanted and the gel was washed successively, with three washing cycles in all, with 0.1 M-sodium acetate buffer, pH 4, containing 1 M-NaCl, and 0.1 M-sodium borate buffer, pH 8.5, containing 1 M-NaCl. The gel was washed overnight by gentle stirring with chloride/phosphate buffer, pH 7.0 (1.7 mM-Na_2HPO_4, 0.52 mM-KH_2PO_4, 44 mM-NaCl) which also contained 0.02% NaN_3. When necessary the gel was stored at 4°C in this azide-containing buffer.

Affinity chromatography

Samples of blood were collected from normal individuals on the day when the experiments were begun. They were allowed to clot for 2 h at 21°C and the serum was separated. The samples of fresh serum (100–200 ml) were dialysed overnight against the chloride/phosphate buffer and then an equal volume of the buffer was added to the serum.

The diluted serum was divided into 30 ml volumes, and each of these was mixed successively for 40 min at 21°C on the roller with a 5 g portion of the IgG–Sepharose 4B preparation. Before each successive addition, the beads were allowed to settle and the supernatant was removed by aspiration. The mixing of the last fraction was usually done overnight.

The beads were washed in a sintered-glass funnel with chloride/phosphate buffer and then poured into a column (5 cm × 2 cm) and eluted with the same buffer at a rate of 50–100 ml/h until after the A_{280} value of the eluate had reached zero (~500 ml of eluting buffer was usually used). The column was operated at 21°C. It was eluted, either with 1% SDS in chloride/phosphate buffer or with 3.5 M-NaCNS solution, at a rate of 12 ml/h. Constant fractions (2–6 ml in different experiments) were collected. Those containing 3.5 M-NaCNS were dialysed exhaustively against water. The absorbances of the fractions at 280 nm were determined, and those fractions containing protein were subjected to polyacrylamide-gel electrophoresis in presence of SDS.

The IgG–Sepharose 4B preparations were re-utilized after washing with large volumes (1000 ml) of phosphate/chloride buffer, and stored in this same buffer with 0.02% sodium azide, at 4°C.

Polyacrylamide-gel electrophoresis in the presence of SDS

This was done by a modification of the procedure of Marshall & Zamecnik (1969). Gels of 7.5% were used, and electrophoresis (0.5–1 mA per gel) was performed at room temperature with 0.1% SDS in

the running buffer (Khalkhali & Marshall, 1976). The samples had been kept at 40°C in the presence of 1% SDS for at least 1 h before electrophoresis.

Crossed immunoelectrophoresis

A modification of the procedure of Laurell (1965) was used, with 1% agarose in sodium barbital/HCl buffer (0.06 M in barbital), pH 8.6. Freeze-dried Tamm–Horsfall glycoprotein (10 μl of a solution, 0.1 mg/ml, in 0.3% SDS) was examined.

Electrophoresis in the first dimension was carried out in gels 9 cm × 2 cm × 0.15 cm at 20 V/cm for 1.5 h, and that in the second dimension for 24 h at 5 V/cm into a 9 cm × 7 cm × 0.15 cm gel containing 0.5% rabbit anti-(human Tamm–Horsfall glycoprotein) serum.

The gel was then washed in 154 mM-NaCl for 24 h and stained with Coomassie Brilliant Blue R250 (0.25% in 1.3 M-acetic acid) for 10 min at 21°C. The gels were destained in acetic acid/methanol/water mixture (38 ml of acetic acid plus 500 ml of methanol made up to a volume of 1 litre with water).

Iodination of Tamm–Horsfall glycoprotein

Tamm–Horsfall glycoprotein was allowed to dissolve in water (1 mg/ml) at 37°C overnight. Portions of this solution were used both for iodination and as the standard in the radioimmunoassay. $Na^{125}I$ solutions (5 μl containing 0.5 mCi) was added to 100 μl of the glycoprotein solution immediately after addition of 50 μl of phosphate buffer, pH 7.4 (19.6 ml of 0.5 M-KH_2PO_4 plus 80.4 ml of 0.5 M-Na_2HPO_4). Chloramine-T solution (50 μl; 4 mg/ml) in Dulbecco A buffer (Dulbecco & Vogt, 1954) was added, the tube was stoppered and the solution was mixed. The reaction was stopped after 30 s by addition of 0.2 ml of sodium metabisulphite solution (4.8 mg/ml in the same buffer). KI (0.4 ml; 20 mg/ml in buffer) was added, followed by 0.2 ml of bovine serum albumin solution (30 mg/ml in buffer). The radiolabelled Tamm–Horsfall glycoprotein was separated on a Sephacryl S-200 column (30 cm × 1.5 cm) equilibrated with Dulbecco A buffer containing 0.02% sodium azide (flow rate 40 ml/h; fraction size 3 ml). The labelled protein fraction was diluted 10-fold in buffer containing bovine serum albumin (2 g/l), and sodium azide (0.02%), and the solution was stored in portions at −20°C for no longer than 4 days. The specific radioactivity was 2.9 μCi/μg (S.D. ± 0.7; $n = 8$). Dilutions for assay were prepared and these contained 100000 c.p.m. per 0.3 ml.

Radioimmunoassay

This was carried out by a modification of the method of Grant & Neuberger (1973).

The standard Tamm–Horsfall glycoprotein solution (1 mg/ml) was serially diluted with buffer containing 0.0011% SDS to give concentrations of 25000, 12500, 6250, 3125, 1560, 781, 390, 195, 98 and 49 ng/ml. The material isolated from serum was serially diluted in a similar way at concentrations ranging from 100000 to 195 mg/ml. All solutions were incubated overnight at 37°C.

Diluted rabbit antiserum (1:2000 in 0.1 M-Tris/HCl buffer, pH 9.5; 0.5 ml) was incubated in polystyrene tubes (40 mm × 11 mm; 1.5 h; 21°C), and the tubes were then washed with two 0.6 ml volumes of Dulbecco A buffer. Bovine serum albumin solution (5 g/l in Dulbecco A buffer) was incubated in the tubes (0.5 h; 21°C) and removed by aspiration. Tamm–Horsfall glycoprotein or serum protein solution (0.25 ml) was then added, followed by 0.3 ml of the radiolabelled solution (100000 c.p.m.). The stoppered tubes were kept at 37°C for 16 h and the solutions were aspirated to remove unbound antigen. After washing the tubes twice (2 × 0.7 ml of water), they were assessed for radioactivity. Non-specific binding was assessed by omission of antibody. All values were less than 1% of the total radioactivity used. All assays were done in duplicate.

Gel filtration of Tamm–Horsfall glycoprotein in the presence of urea

This was carried out on a column (80 cm × 2.3 cm) of Bio-Gel A.15m, by the procedure of Dawnay *et al.* (1980).

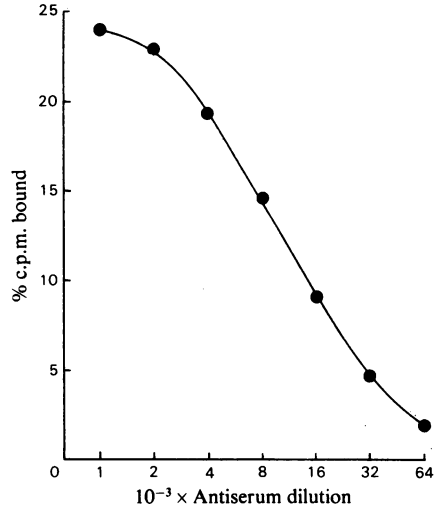

Fig. 1. *Antiserum dilution curve*
An amount of radiolabelled Tamm–Horsfall glycoprotein equivalent to 100000 c.p.m. was used.

Results

Crossed immunoelectrophoresis of Tamm–Horsfall glycoprotein antiserum revealed the presence of a single precipitin line. An antiserum titration curve is shown (Fig. 1). A dilution of antiserum of 1:2000 was used to coat the tubes in the radioimmunoassay.

Affinity chromatography of 200ml of dialysed human serum, in a system in which Sepharose 4B linked to IgG raised in rabbits against human Tamm–Horsfall glycoprotein was used as the affinity material, led to binding of only a few mg of the greater than 10g of protein in the original serum. After thorough washing of the adsorbent, with the phosphate/chloride buffer, until after A_{280} of the

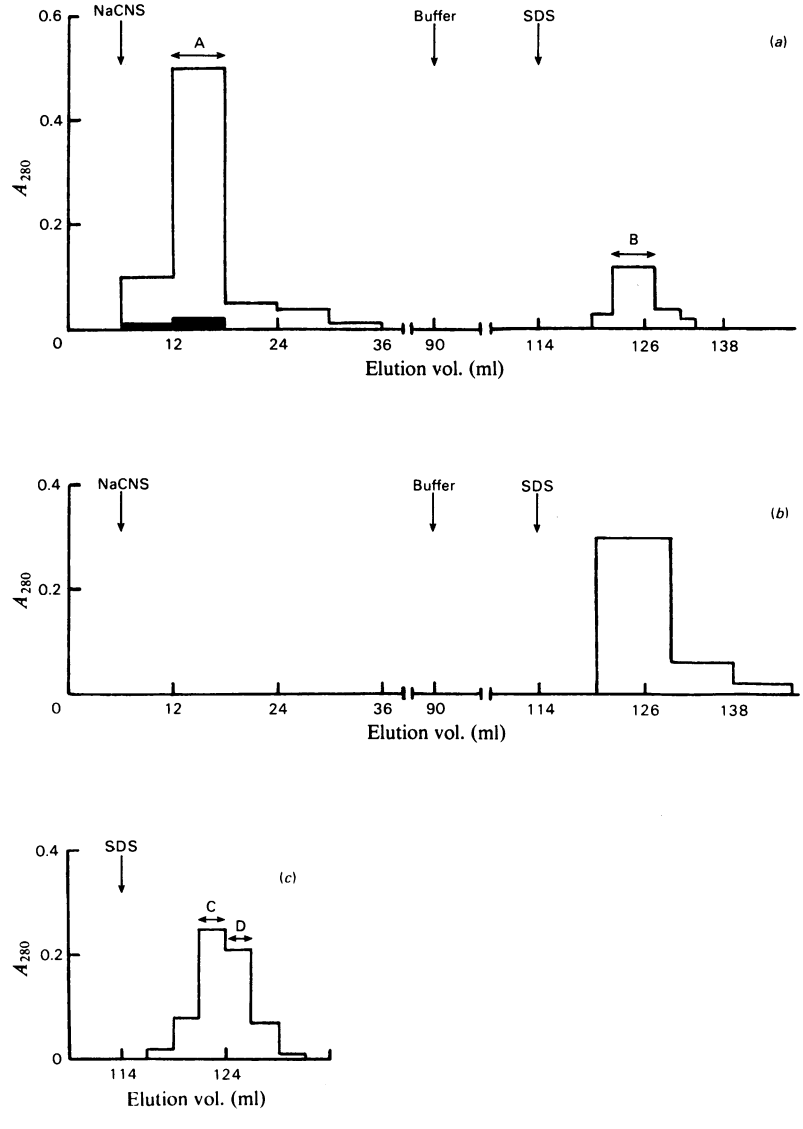

Fig. 2. *Affinity chromatography with rabbit-(anti-human Tamm–Horsfall glycoprotein)IgG*
For details see the text. (*a*) 200ml of pooled, normal human serum, (*b*) 2mg of freeze-dried Tamm–Horsfall glycoprotein dissolved in 100ml of chloride/phosphate buffer and (*c*) 150ml of pooled, normal human serum. The points at which the eluting agent was changed are indicated by arrows: 3.5M-NaCNS (NaCNS), the chloride/phosphate buffer (Buffer) and 1% SDS (SDS). The shaded area indicates the absorbance in fractions from the control column (see the text).

eluate was zero, the column was prepared and was eluted with 3.5 M-NaCNS solution (pH 6.8). An amount of 4.02 mg of protein was found, on the assumption that a concentration of 1 mg/ml gives an absorbance of unity. A further smaller amount of protein (0.78 mg) was eluted with 1% SDS (Fig. 2*a*). The total amount recovered was 24 mg/l of serum.

The material in zone A (Fig. 2*a*) revealed two bands upon polyacrylamide-gel electrophoresis in the presence of SDS (Fig. 3*a*). The mobility of the faster one (F) was greater than that of Tamm–Horsfall glycoprotein. The mobility of the second band (S) was less than that of the latter but greater than that of sheep IgG. Material in zone B (Fig. 2*a*) was shown to have the same mobility as that in band F (Fig. 3*b*).

In a control experiment 130 ml of human serum was subjected to affinity chromatography with material made from IgG isolated from the serum of rabbits which had not been immunized. The column was eluted successively with the chloride/phosphate buffer (to elute the unadsorbed serum protein), 3.5 M-NaCNS, chloride/phosphate buffer and 1% SDS. Negligible amounts of protein were found in the dissociating eluants (Fig. 2*a*).

Tamm–Horsfall glycoprotein exhibits different behaviour from proteins S and F upon affinity chromatography. The glycoprotein (2 mg) dissolved in 100 ml of buffer was subjected to chromatography in the manner used for serum. It could not be eluted from the column by 3.5 M-NaCNS, but a recovery of the order of 70% was achieved by the use of 1% SDS (Fig. 2*b*). It is relevant to mention that Tamm–Horsfall glycoprotein is largely present in a polymeric form in solution in 3.5 M-NaCNS, because it was found to be eluted from a Sephacryl S-200 column in this solvent with a V_e/V_0 value of 1.05. On the other hand it exists in its subunit form in 1% SDS solutions (Fletcher *et al.*, 1970).

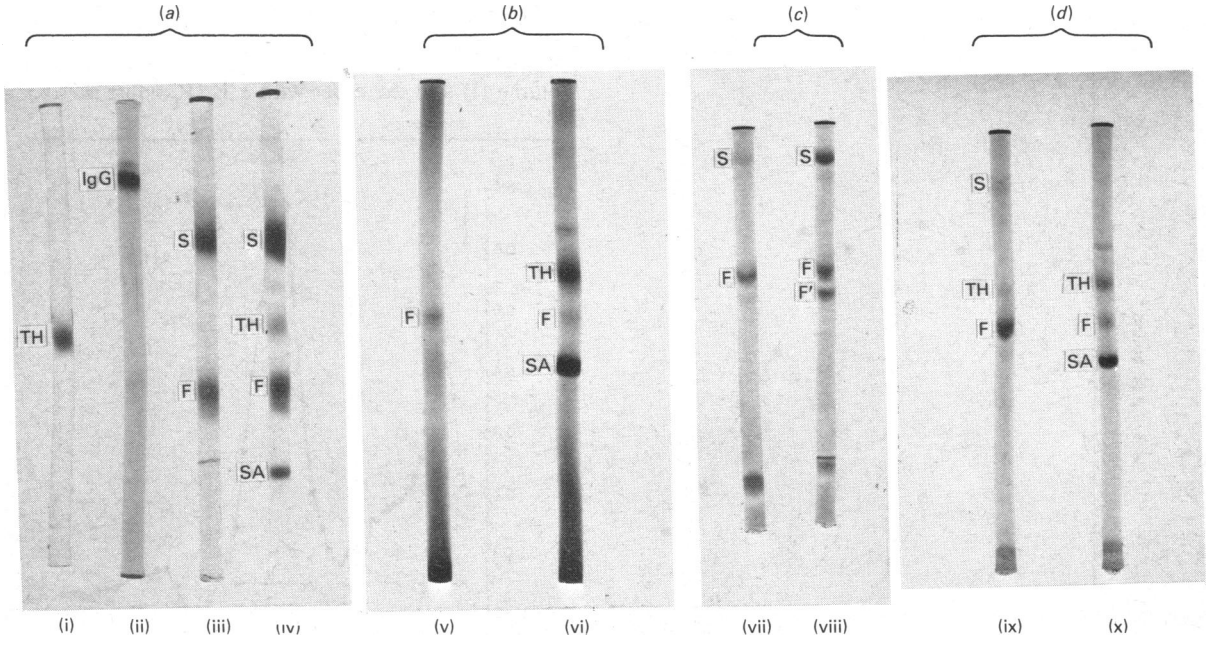

Fig. 3. *Polyacrylamide disc-gel electrophoresis in the presence of SDS*

(*a*): (i) 15 μg of Tamm–Horsfall glycoprotein (TH); (ii) 15 μg of sheep IgG; (iii) 50 μg of serum protein from zone A (Fig. 2*a*); (iv) a mixture of 50 μg of serum protein from zone A, 10 μg of Tamm–Horsfall glycoprotein and 10 μg of human serum albumin (SA). The marker dye was allowed to run off the end of the gel. (*b*): (v) 15 μg of serum protein from zone B (Fig. 2*a*); (vi) a mixture of 15 μg of serum protein from zone B, 10 μg of Tamm–Horsfall glycoprotein and 10 μg of human serum albumin. The slowest moving weak band arises from the human serum albumin. The gels were cut at the dye front. (*c*): (vii) 15 μg of serum protein from zone C (Fig. 2*c*); (viii) 15 μg of serum protein from zone D (Fig. 2*c*). The marker dye, incompletely destained, is seen near the bottom of the gels. (*d*): (ix) 15 μg of protein eluted by 1% SDS from the affinity column after application of a mixture of 1.4 mg of Tamm–Horsfall glycoprotein and 175 ml of dialysed serum; (x) a mixture of the protein as in (ix) with added human serum albumin (10 μg) and Tamm–Horsfall glycoprotein (10 μg). The slowest moving weak band arises from the human serum albumin. The marker dye, incompletely destained, is seen near the bottom of the gels.

Elution of serum protein which bound to the affinity material was also effected directly with SDS. Interaction of 150ml of serum with anti-(Tamm–Horsfall)-IgG–Sepharose 4B was followed by elution with chloride/phosphate buffer and then by 1% SDS. A total amount of protein of 2.9mg was eluted by the detergent (Fig. 2*c*), equivalent to 19mg/l of serum. Polyacrylamide-gel electrophoresis of the peak fraction (zone C) showed the presence of proteins F and S (Fig. 3*c*). In addition, in one of the later fractions (zone D), there was a further band clearly seen (F^1). The mobilities relative to Bromophenol Blue were for cytochrome *c* (0.95), hen's egg albumin (0.56), human serum albumin (0.45), Tamm–Horsfall glycoprotein (0.31), sheep IgG (0.08), protein F^1 (0.41), protein F (0.37) and protein S (0.14). Apparent mol.wts. may be calculated as 74000, 84000 and about 125000 for F^1, F and S respectively, bearing in mind, that in this system of electrophoresis, Tamm–Horsfall glycoprotein behaves as a protein with an apparent mol.wt. of 105000 (Fletcher *et al.*, 1970).

A similar experiment was done on a mixture of 175ml of dialysed serum to which had been added 1.4mg of Tamm–Horsfall glycoprotein before interaction with the affinity gel. Elution with 1% SDS, subsequent to elution of the bulk of the protein with the chloride/phosphate buffer, yielded an elution pattern like that shown in Fig. 2(*c*). A total of 3.9mg of protein was found. Polyacrylamide-gel electrophoresis in the presence of SDS of the peak fraction revealed that protein F and Tamm–Horsfall glycoprotein were present (Fig. 3*d*), as distinct materials.

Material (zone A, Fig. 2*a*) isolated by affinity chromatography with the use of 3.5M-NaCNS was subjected to radioimmunoassay after exhaustive dialysis against water followed by Dulbecco A buffer containing 0.0011% SDS. The substance isolated from serum was found to have a much lower affinity for the antibody than did Tamm–Horsfall glycoprotein (Fig. 4). About 60–70 times as much of the substances in serum are required as of freeze-dried Tamm–Horsfall glycoprotein to compete with a given amount of the radiolabelled form of the latter.

Urinary Tamm–Horsfall glycoprotein, which had been subjected to gel filtration in the presence of urea, gave two protein peaks (Fig. 5) [one at the void volume (I) and the other with a V_e/V_0 value of 1.83

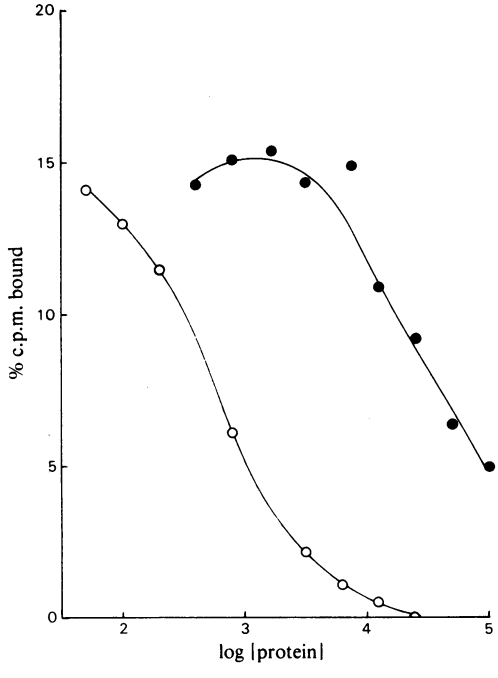

Fig. 4. *Radioimmunoassay of Tamm–Horsfall glycoprotein standard* (○) *and of the immunologically cross-reactive material derived from serum in zone A of Fig. 2a* (●)

The concentrations of protein in the two cases are shown on the abscissa as ng/ml. The radiolabel for both curves was ^{125}I-labelled Tamm–Horsfall glycoprotein and the assays for the urinary and serum derived proteins were done in the same experiment.

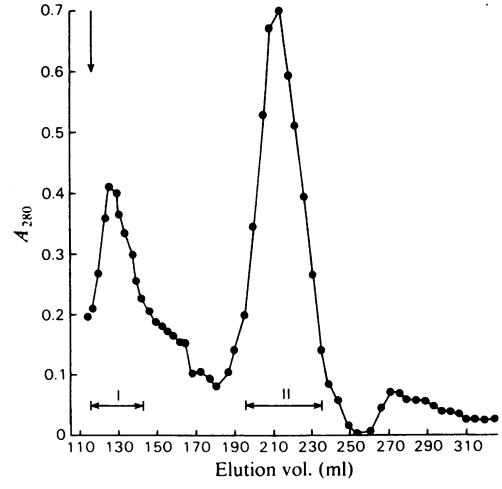

Fig. 5. *Gel filtration of Tamm–Horsfall glycoprotein on Bio-Gel A.15m (80cm × 2.3cm) at 21°C, in 0.03M-sodium phosphate buffer, pH6.8, containing 2M-urea*

The glycoprotein (35mg) had previously been dissolved in 5ml of 8M-urea in the same buffer for 16h at 21°C. The column was eluted at 7ml/h. The void volume of the column is indicated by an arrow. Pooled fractions I and II were individually dialysed against water and freeze-dried. Amounts of 5.5 and 25mg respectively were found (85% recovery). The elution volume for bovine serum albumin was 222ml.

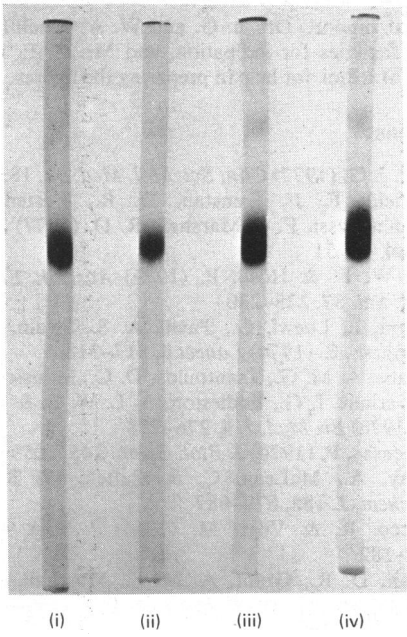

Fig. 6. *Comparison of the mobilities upon polyacrylamide electrophoresis in the presence of SDS*
(i) 20 μg of freeze-dried urinary Tamm–Horsfall glycoprotein, (ii) 30 μg of pooled fractions I (Fig. 5), (iii) 30 μg of pooled fractions II (Fig. 5) and (iv) a mixture of 15 μg of Tamm–Horsfall glycoprotein and 30 μg of pooled fractions II (Fig. 5).

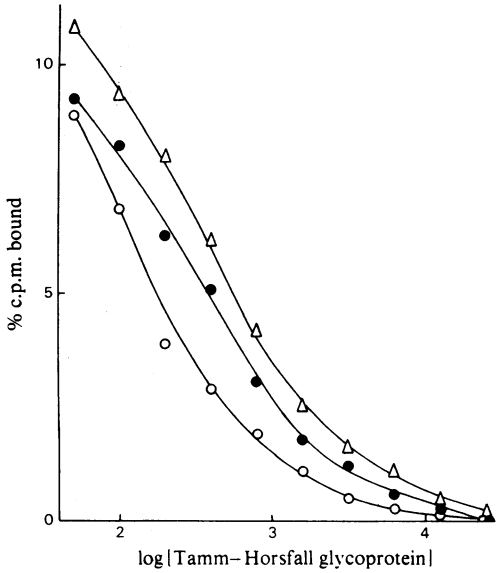

Fig. 7. *Radioimmunoassay of freeze-dried urinary Tamm–Horsfall glycoprotein standard* (●), *of pooled fraction I in Fig. 5* (△) *and of pooled fraction II in Fig. 5* (○)
The radiolabel was ^{125}I-labelled freeze-dried urinary Tamm–Horsfall glycoprotein and all measurements were made in the same experiment. The concentration of the protein is expressed as ng/ml.

(II)] with electrophoretic mobilities in the presence of SDS unaltered from that of the original material (Fig. 6). The mobility was different from those of proteins S and F.

Radioimmunoassay of fractions I, II and of the glycoprotein itself were carried out by using the same preparation of radiolabelled glycoprotein. The results showed (Fig. 7) that about three times as much of the aggregated form of the urea-treated glycoprotein (I, Fig. 5) as of the disaggregated material (II) was required to compete with a given amount of the radiolabelled glycoprotein.

Discussion

There are present, in normal human serum, three proteins which we have called S, F and F[1], which will bind to IgG isolated from the serum of rabbits immunized with human urinary Tamm–Horsfall glycoprotein. The proteins have apparent molecular weights, as assessed by disc-gel electrophoresis in the presence of SDS, of 125000, 84000 and 74000 respectively. The mobility of each of these differs from that of Tamm–Horsfall glycoprotein, the presence of which could not be demonstrated in normal serum.

In addition, the proteins S and F differ from Tamm–Horsfall glycoprotein in that their complexes with Sepharose 4B-linked antibody can be dissociated in 3.5 M-NaCNS. This reagent, in which Tamm–Horsfall glycoprotein exists in an aggregated form, does not dissociate the urinary glycoprotein–antibody complex.

The cross-reacting serum proteins compete with radiolabelled Tamm–Horsfall glycoprotein in the immunoassay, although they are bound less avidly than is the latter. About 60–70 times as much of the serum proteins are required as of Tamm–Horsfall glycoprotein to compete with a given amount of ^{125}I-labelled urinary glycoprotein.

The cross-reacting serum proteins were isolated in amounts of 19–24 mg/l of serum, and it is likely that this represents a substantial proportion of that originally present, in view of the recovery found in the present work (70%) for Tamm–Horsfall glycoprotein itself (see also Dunstan *et al.*, 1974). Much smaller quantities (0.05–0.18 mg/l, Avis, 1977; 0.07–0.54 mg/l, Dawnay *et al.*, 1980) of a substance, that reacted similarly to Tamm–Horsfall glycoprotein in a radioimmunoassay, were reported to be present in serum, but no account was taken of the immunoreactivity of the material. Possibly the substance measured by Avis (1977) and by Dawnay *et al.* (1980) was not Tamm–Horsfall glycoprotein but was the cross-reacting protein which we have isolated. Their values, with appropriate correction for the differences in reactivity, would become about 3–12 mg/l and 5–35 mg/l respectively which are of

the same order as we have found for the cross-reacting material.

It was reported by Dawnay *et al.* (1980) that gel filtration of Tamm–Horsfall glycoprotein in the presence of urea, under the conditions used in the present experiments (Fig. 5), yielded a product, which was more mobile upon polyacrylamide-gel electrophoresis in the presence of SDS than was the original glycoprotein. The precise results on which this claim was made were not given, but in our experience this report is erroneous. The electrophoretic mobilities of both fractions from the Bio-Gel column (Fig. 5) and of Tamm–Horsfall glycoprotein itself were identical (Fig. 6) and their immunoreactivities were similar (Fig. 7). Dawnay *et al.* (1980) deduced from their reported findings that normal human serum contains a monomeric form of urinary Tamm–Horsfall glycoprotein. Our results would suggest that this is not so.

The present findings raise many questions. The serum proteins require characterization in order to understand the basis of the immunological cross-reactivity with Tamm–Horsfall glycoprotein. The identity of the substance associated with the hepatocyte plasma membrane, which interacts weakly with antibody raised against Tamm–Horsfall glycoprotein (Tsantoulos *et al.*, 1974), requires elucidation and the results might help to explain why a relatively large number of patients with autoimmune hepatic disease (32% in one study; Golding *et al.*, 1973) have an associated renal tubular acidosis. The nature of the substance, cross-reactive with Tamm–Horsfall glycoprotein and produced by a line of cultured kidney cells (Bloomfield *et al.*, 1977), is presently uncertain.

The antigen involved in the formation of antibodies, which interact with Tamm–Horsfall glycoprotein *in vitro* in those with acute pyelonephritis (Fasth *et al.*, 1979), or with cystic fibrosis (Fasth & Kollberg, 1980), is unknown. Whether the antibodies produced react also with the serum protein is problematical. Clarification of the nature of the antibodies, demonstrated by indirect immunofluorescence, reactive with the epithelium of the cortical ascending limb of the loop of Henle and of the distal convoluted tubule and present in the sera of patients with disorders presumed to be of immune aetiology (Pasternak & Linder, 1970; Ford, 1973; Chanarin *et al.*, 1974) is needed. Further investigation of the nature of the factor produced by cultured normal fibroblasts, that inhibits induction, by Tamm–Horsfall glycoprotein, of alkaline phosphatase in fibroblasts derived from cystic fibrosis patients (Carey & Hösli, 1979), might yield interesting information in the light of our findings.

We thank the Wellcome Trust, the Medical Research Council and the National Kidney Research Fund for financial support, Drs. J. G. and W. A. Ratcliffe for the use of facilities for iodination, and Mr. C. E. L. Shaw, M.Sc., M.I.Biol. for help in preparing the figures.

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