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Effects of experimental nephrosis on basement-membrane components and enzymes of collagen biosynthesis in rat kidney

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The aim of the present study was to find out whether the basement-membrane proteins laminin and type IV collagen are involved in the development of aminonucleoside-induced nephrosis. These proteins were measured by specific radioimmunoassays in serum, urine and kidney-cortex samples, and they were localized in the glomeruli by indirect immunofluorescence. Nephrosis was induced in rats with a single intraperitoneal injection of puromycin aminonucleoside. Serum laminin concentrations, detected by a radioimmunoassay for the P2 domain of the protein, increased to reach a maximum at days 5–7, and they remained elevated until at least day 14. The increase preceded the development of proteinuria, suggesting a role for laminin in glomerular function. Concomitant with proteinuria, increasing amounts of laminin antigenicity were also found in the urine. The size of the laminin antigen in serum was estimated by gel filtration, and the serum forms were found to contain both the P1 and the P2 regions of the intact laminin molecule. On the other hand, there were no changes in the serum or urinary concentrations of type-IV-collagen-derived antigens, as detected by a radioimmunoassay for the 7S collagen domain of this protein. The total content of laminin in kidney cortex, measured after digestion of the tissue with trypsin and collagenase, was, at day 9, still comparable with normal values, and the distribution of both basement-membrane proteins in the glomeruli, studied by indirect immunofluorescence, was similar to that in the controls. The tissue damage induced by aminonucleoside, however, seems to stimulate collagen biosynthesis, as the activities of prolyl 4-hydroxylase, lysyl hydroxylase and galactosylhydroxylysyl glucosyltransferase in kidney tissue increased significantly, with maxima at days 8–10.

The aminonucleoside of puromycin, PAN, is widely used for induction of proteinuria in experimental animals, thus simulating either the minimal-change nephropathy (Vernier *et al.*, 1959) or the focal sclerosis (Glasser *et al.*, 1977) found in man. However, the mechanism by which this nucleotide analogue at the molecular level brings about the changes in glomerular permeability is still unknown, although the function and the histological appearance of such diseased glomeruli have been studied extensively.

In the experimental nephrotic syndrome induced by PAN, many authors (Michael *et al.*, 1970; Caulfield & Farquhar, 1978), though not all (Kanwar & Jakubowski, 1984), have found an apparent decrease in fixed anionic sites in the glomerular capillary wall, leading to increased permeability for negatively charged proteins (Bohrer *et al.*, 1977; Olson *et al.*, 1981). In addition, the size-selective properties of the filtration barrier are modified (Olson *et al.*, 1981). Among the ultrastructural lesions seen are a partial or complete detachment of the epithelium from the GBM (Caulfield *et al.*, 1976) and extensive retraction of the epithelial-cell foot processes (Michael *et al.*, 1970; Caulfield *et al.*, 1976). It has been suggested that during the development of a PAN nephrosis lysosomal enzymes are liberated that could digest part of the extracellular matrix, resulting in these changes (Velosa *et al.*, 1981). Also, histamine has been suggested to be involved in altering glomerular

Abbreviations used: PAN, puromycin aminonucleoside; GBM, glomerular basement membrane.

capillary permeability in this condition (Abboud *et al.*, 1982).

The nephrotic state caused by a single injection of PAN in rats usually subsides in about 3 weeks (Glasser *et al.*, 1977). However, repeated administration of PAN results in a progressive kidney disease characterized by focal sclerotic changes in the glomeruli and leading to permanent renal failure (Glasser *et al.*, 1977).

GBM is considered the main site of filtration in the glomerulus (Brenner *et al.*, 1978). Chemically its most abundant constituent is a collagen, which is genetically distinct from the interstitial collagens and has been termed type IV collagen (Bornstein & Sage, 1980). Type IV collagen does not form fibrils, and it has been suggested that type IV collagen molecules are organized into a net-like structure (Timpl *et al.*, 1981). In this net the individual molecules would be linked to each other at both ends. The *N*-terminal domain of type IV collagen, known as the 7S domain (J. Risteli *et al.*, 1980), is markedly resistant both to thermal denaturation and to proteolytic digestion. The non-collagenous components of the GBM include laminin (Timpl *et al.*, 1979), which has been shown to mediate the attachment of several cell types to a matrix, fibronectin (Stenman & Vaheri, 1978), entactin (Carlin *et al.*, 1981), nidogen (Timpl *et al.*, 1983), and a heparan sulphate-containing proteoglycan (Kanwar & Farquhar, 1979; Hassell *et al.*, 1980). In spite of its small amount in the GBM, the proteoglycan probably provides a significant part of the anionic sites in GBM.

It has been shown by immunohistochemical methods that the content of heparan sulphate proteoglycan in GBM is decreased in PAN nephrosis (Mynderse *et al.*, 1983). On the other hand, a decreased content of collagen-specific amino acids in GBM has been reported in nephrosis (Kefalides & Forsell-Knott, 1970), and nephrotic GBM is mechanically more fragile than normal GBM (Krakower *et al.*, 1978), suggesting profound alterations in its composition. The aim of the present study was to characterize the changes that PAN brings about in the metabolism of collagen and basement membrane in rat kidney cortex by using specific immunological methods and by assessing the activity of several collagen-modifying enzymes.

Materials and methods

Animals and induction of nephrosis

Female Sprague–Dawley rats, weighing 200–300g, from the Department's own stocks were used. They were housed in individual metabolic cages and had free access to commercial rat chow and tap water during the whole experiment. Nephrosis was induced at day 0 by a single intraperitoneal injection of PAN (Sigma Chemical Co., St. Louis, MO, U.S.A.), by using 15mg of the drug/100g body wt. (Olson *et al.*, 1981; Velosa *et al.*, 1981). PAN was dissolved in iso-osmotic (0.9%) NaCl, and control rats were injected with an equivalent volume of iso-osmotic NaCl. Urine samples (24h) were collected daily, into beakers containing 100μl of 6M-HCl to prevent bacterial growth, and the samples were stored frozen at -20°C until assayed. At the times indicated (see the Results section) the rats were anaesthetized with diethyl ether, blood was collected by cardiac puncture and the kidneys were removed. The kidneys were immediately frozen in liquid N_2 and kept frozen until assayed.

Radioimmunoassays

Mouse laminin and the 7S domain of type IV collagen were purified from transplantable mouse EHS tumours (Timpl *et al.*, 1979; J. Risteli *et al.*, 1980), and P1 and P2 fragments were prepared from laminin by gel filtration after pepsin digestion (Rohde *et al.*, 1980). Antisera were raised in rabbits as described previously (Timpl *et al.*, 1979; J. Risteli *et al.*, 1980). The antigens were labelled with ^{125}I by the chloramine-T method (Timpl & Risteli, 1982). In the radioimmunoinhibition assay (sequential saturation type), 0.1 ml of an antiserum dilution capable of binding 50% of the labelled antigen was incubated for 16h at +4°C together with 0.2ml of non-labelled antigen solution or unknown sample. After addition of 1 ng of labelled antigen (25000d.p.m. in 0.1 ml), incubation was continued for 6h. The free and bound antigens were then separated by precipitation (16h at +4°C) with 0.01 ml of goat antiserum to rabbit IgG and the radioactivities in the precipitates were counted in an LKB Multigamma counter. All dilutions were made in phosphate-buffered saline (Timpl & Risteli, 1982), pH 7.2, containing 0.04% Tween 20 to minimize non-specific binding and 0.02% NaN_3 to prevent bacterial growth. The antiserum dilutions (range 1:800 to 1:8000) contained 0.5% of normal rabbit serum as carrier. Non-specific binding of the labelled antigen to the carrier was in the range of 1–4%. A concentration of about 5ng of the standard antigen/ml gave 50% inhibition in the assays used. The coefficient of variation within the assay was 2–10% and that between the assays was 15–25% (Risteli *et al.*, 1981). All the samples included in the same Table or Figure were analysed simultaneously. Before radioimmunoassay, the urine samples were dialysed at +4°C against phosphate-buffered saline, pH 7.2, containing Tween 20 and NaN_3.

Proteolytic digestion of kidney cortices

Pieces of kidney cortex (about 100mg wet wt.) were homogenized in a Teflon/glass homogenizer in a cold (+4°C) solution (2ml/100mg of tissue) containing 1% Triton X-100, 10mM-EDTA and the proteinase inhibitors *p*-hydroxymercuribenzoate (50μg/ml) and phenylmethanesulphonyl fluoride (50μg/ml), pH 7.2. The homogenate was centrifuged at 15000*g* for 10min, the supernatant discarded and the precipitate washed once with an equal volume of water. The precipitate was then suspended in 0.2M-NH_4HCO_3, pH 7.9 (2ml/100mg original tissue wt.), and trypsin [treated with the chymotrypsin inhibitor 1-chloro-4-phenyl-3-L-tosylamidobutan-2-one ('TPCK'); Worthington, Freehold, NJ, U.S.A.; 80μg/100mg of tissue] and collagenase (highly purified collagenase; Worthington; 16μg/100mg of tissue) were added. Incubation was carried out at +20°C for 6h. Then soya-bean trypsin inhibitor was added (Sigma Chemical Co.; 80μg/100mg of tissue) and the samples were centrifuged to remove the small insoluble residue. The supernatants were stored at −20°C until assayed.

Indirect immunofluorescence

Antibodies against human 7S collagen (J. Risteli *et al.*, 1980), human laminin fragment P1 (Risteli & Timpl, 1981) and intact mouse laminin (Timpl *et al.*, 1979) were prepared in rabbits as described previously, and purified by immunoabsorption on the relevant antigen coupled to Sepharose 4B (Furthmayr, 1982). Laminin antibodies were cross-absorbed with type IV collagen and vice versa, and there were no cross-reactions in the radioimmunoassays for the two antigens.

Frozen unfixed tissue sections (4μm thick) were used in indirect immunofluorescence tests performed by a standard procedure (Wick *et al.*, 1978). The antibodies were used at concentrations of 5–30μg/ml, and fluorescein isothiocyanate conjugates of antisera to rabbit IgG (Dako, Copenhagen, Denmark) were used at a dilution of 1:20.

Gel filtration of serum samples

Samples (2ml) were chromatographed on a column (1.5cm × 100cm) of Sephacryl S-300 (Pharmacia Fine Chemicals AB, Uppsala, Sweden) equilibrated in phosphate-buffered saline, pH 7.2, containing 0.04% Tween 20; 2.5ml fractions were collected.

Activities of the intracellular enzymes of collagen biosynthesis

For the enzyme assays, pieces of kidney tissue were homogenized in a Teflon/glass homogenizer in a cold (+4°C) solution containing 0.2M-NaCl, 0.1M-glycine, 0.1% Triton X-100, 0.01% soya-bean trypsin inhibitor and 0.02M-Tris/HCl buffer, pH adjusted to 7.5 at +4°C (1ml/300mg of tissue) (Kivirikko & Myllylä, 1982). After 30min at +4°C, the homogenates were centrifuged at 15000*g* for 30min at +4°C, and samples of the supernatants were assayed immediately for the intracellular enzyme activities.

The assay for prolyl 4-hydroxylase activity was carried out by incubating portions of the supernatants under agitation for 30min at +37°C in a final volume of 1ml containing 50000d.p.m. of [^{14}C]proline-labelled protocollagen substrate, 0.08mM-$FeSO_4$, 2mM-ascorbic acid, 0.5mM-2-oxoglutarate, 0.2mg of catalase (Calbiochem, San Diego, CA, U.S.A.), 2mg of bovine serum albumin (Sigma Chemical Co.) and 50mM-Tris/HCl buffer, pH adjusted to 7.8 at +25°C (Kivirikko & Myllylä, 1982). The reaction was stopped by adding 1ml of concentrated HCl, and the amount of hydroxy-[^{14}C]proline formed was measured after hydrolysis at +120°C overnight (Kivirikko & Myllylä, 1982).

In the assay for lysyl hydroxylase activity, portions of the supernatants were incubated under agitation for 45min at 37°C in a final volume of 1ml containing 200000d.p.m. of [6-^{3}H]lysine-labelled protocollagen substrate (prepared as described by L. Risteli *et al.*, 1980), 0.08mM-$FeSO_4$, 2mM-ascorbic acid, 0.5mM-2-oxoglutarate, 0.2mg of catalase, 2mg of bovine serum albumin and 50mM-Tris/HCl buffer, pH adjusted to 7.8 at +25°C (Kivirikko & Myllylä, 1982). The reaction was stopped by adding 10ml of cold acetone, and the radioactive hydroxylysine formed was measured (Kivirikko & Myllylä, 1982). If degradation of the substrate during the incubation was also to be tested, the acetone supernatant obtained after centrifugation was air-dried, dissolved in water and measured for ^{3}H radioactivity.

To assay hydroxylysyl galactosyltransferase activity, portions of the 15000*g* supernatants were incubated under agitation in a final volume of 100μl containing 34mg of gelatinized collagen substrate/ml, 60μM-UDP-galactose (New England Nuclear Corp., Boston, MA, U.S.A.; diluted with the unlabelled compound to a final specific radioactivity of 10Ci/mol), 0.01M-$MnCl_2$, 0.8M-NaCl and 50mM-Tris/HCl buffer, pH adjusted to 7.4 at +37°C (Kivirikko & Myllylä, 1982). Galactosylhydroxylysyl glucosyltransferase activity was assayed in a similar manner, but 60μM-UDP-glucose (New England Nuclear Corp.; 3.3Ci/mol) was substituted for UDP-galactose (Kivirikko & Myllylä, 1982). The reactions were carried out for 45min at +37°C and stopped by adding 2ml of 1% phosphotungstic acid in 0.5M-HCl, and the reaction products were assayed as reported previously (Kivirikko & Myllylä, 1982).

Other assays

Urinary and serum protein content was determined by a modification of the biuret method (Hiller *et al.*, 1949).

Statistical methods

The statistical significance of the differences between means was calculated by Student's t test.

Results

Nephrotic state of the animals

Nephrosis was induced with a single injection of PAN. This resulted in a highly reproducible pattern of proteinuria (see Fig. 3), with a maximum at day 9 (Olson *et al.*, 1981; Abboud *et al.*, 1982). At this time all the nephrotic animals had a marked proteinuria and significant hypoproteinaemia (Table 1). The weight of the nephrotic rats was less than that of the controls, and the kidneys were enlarged (Table 1).

Enzymes of collagen biosynthesis in kidney tissue

In order to study the effect of the nephrosis on the metabolism of collagen in kidney tissue, the activities of four intracellular enzymes of collagen biosynthesis were measured in the 15000g supernatants of kidney homogenate at days 3–14 (Fig. 1). All of these activities (prolyl 4-hydroxylase, lysyl hydroxylase, hydroxylysyl galactosyltransferase and galactosylhydroxylysyl glucosyltransfer-

Table 1. *Characterization of the nephrotic state of the rats at day 9 after administration of PAN*
Each group contained six rats. For details see the Materials and methods section. Values are expressed as means ± S.D. NS, not significant.

	Controls	PAN-induced nephrosis	P
Body wt. (g)			
at day 0	288 ± 15	289 ± 14	NS
at day 9	296 ± 18	270 ± 20	<0.05
Kidney wt. (g)	1.99 ± 0.17	2.68 ± 0.25	<0.001
Serum protein (g/dl)	9.07 ± 0.45	7.04 ± 0.36	<0.001
Urine flow (ml/24h)	9.25 ± 5.95	18.8 ± 8.84	<0.05
Urine protein (mg/24h per 100g body wt.)	9.9 ± 4.0	228.8 ± 63.5	<0.001

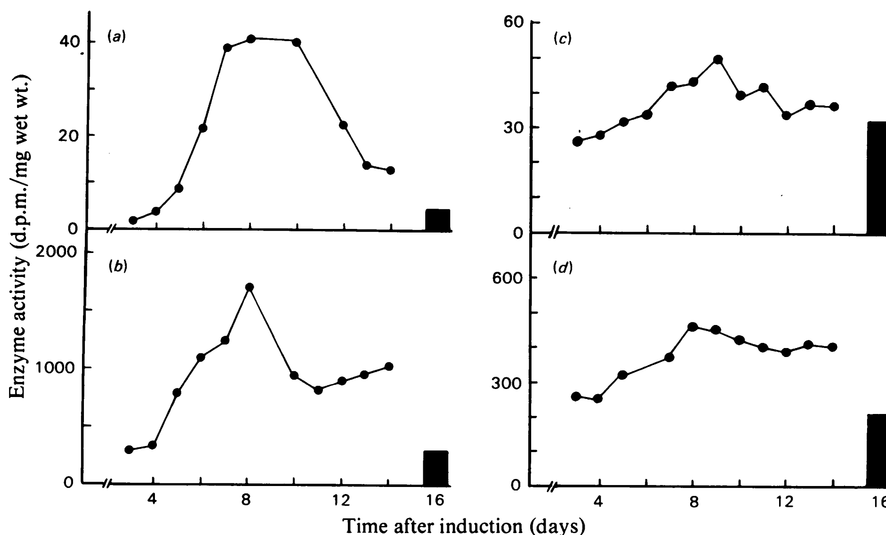

Fig. 1. *Activities of four intracellular enzymes of collagen biosynthesis in kidney cortex as a function of time after administration of PAN*

The values are expressed as radioactivity (d.p.m.) of product formed/mg wet wt. of tissue. Each point represents the mean for two animals. The column indicates the mean value for the control animals. (*a*) Lysyl hydroxylase; (*b*) prolyl-4-hydroxylase; (*c*) hydroxylysyl galactosyltransferase; (*d*) galactosylhydroxylysyl glucosyltransferase.

ase) were very low in normal rat kidney. The induction of nephrosis caused a marked increase in the activities of the two hydroxylases. The maximal values, reached at day 9, were 3.5 and 20 times those in the controls for prolyl 4-hydroxylase and lysyl hydroxylase respectively (Table 2). The amount of the radioactive protocollagen substrate that was degraded into small peptides no longer precipitable with acetone in the lysyl hydroxylase assay was somewhat larger in the controls than in the nephrotic samples, but was in both cases less than 10% of the total radioactivity present in the substrate.

In contrast with the hydroxylases, there were only slight increases in the activities of the collagen glycosyltransferases during development of the nephrosis (Fig. 1). However, the about 2-fold increase in the glucosyltransferase activity at day 9 was statistically highly significant (Table 2).

After the maximal values all the elevated enzyme activities showed a decrease, but did not reach the control region by day 14 (Fig. 1).

Type IV collagen and laminin in kidney cortex

In routine histological analysis there were no differences between the kidney cortices of nephrotic and normal rats (results not shown). Laminin and type IV collagen were localized in the basement membranes of the glomerulus by indirect immunofluorescence, the distribution in the nephrotic rats being similar to that in the controls (Fig. 2).

In order to quantify laminin in the tissue, pieces of kidney cortex were exhaustively treated with trypsin and collagenase, and the laminin P2 antigen released was measured by radioimmunoassay. The concentrations were 9.95 ± 1.64 and 9.47 ± 1.83 ng of laminin P2 antigen/mg wet wt. of tissue for the controls and the nephrotic animals respectively. The amounts of 7S collagen antigen released were 27.8 ± 2.58 and 25.4 ± 3.18 ng/mg wet wt. of tissue respectively, but the proteolytic treatment used here evidently cannot solubilize this collagen completely from the tissue (Risteli *et al.*, 1981), although it is optimal for the extraction of laminin P2 antigen.

Type IV collagen and laminin in serum and urine

The concentration of basement-membrane-derived proteins in serum and urine were measured by specific radioimmunoassays for the P2 domain of laminin (Rohde *et al.*, 1980; Ott *et al.*, 1982) and the 7S collagen domain of type IV collagen (J. Risteli *et al.*, 1980). The serum concentration of laminin P2 antigen increased to reach a maximum as early as on days 5–7 and decreased after the onset of polyuria on day 8 (Fig. 3). However, it remained clearly elevated until at least day 14. In contrast, there was no difference in the mean serum concentration of 7S collagen between the nephrotic and control rats. The amount of 7S collagen antigen in the urine was below the detection limit of the radioimmunoassay for both normal and nephrotic rats, the excretion of this antigen thus being less than 0.15 ng/24 h per 100 g body wt. The concentration of the laminin P2 antigen was only just measurable in normal rat urine, but by day 9 of nephrosis there was a 16-fold increase in laminin excretion (2.4 ± 1.1 and 40 ± 18 ng/24 h per 100 g respectively; $P < 0.001$).

During a follow-up experiment, the urinary excretion of the laminin P2 antigen was found to resemble that of total protein (Fig. 3*b*), a maximum, however, being reached somewhat later than the peak of protein excretion.

Characterization of the serum laminin antigen

To characterize the serum antigen related to laminin, sera from both control and nephrotic rats at day 9 were subjected to gel filtration on a Sephacryl S-300 column (Fig. 4). In normal rat serum three peaks of laminin P2 antigenicity were detected, one eluted near the exclusion volume of the column and also the second representing material

Table 2. *Activities of intracellular enzymes of collagen biosynthesis in kidneys at day 9 after administration of PAN*
Each group contained six animals. The enzyme activities (for details see the Materials and methods section) are expressed as radioactivity (d.p.m.) of product formed/mg wet wt. of tissue. Values are expressed as means ± S.D. NS, not significant.

Enzyme	Activity (d.p.m./mg wet wt.) Controls	PAN-induced nephrosis	P
Prolyl 4-hydroxylase	516 ± 187	1834 ± 201	<0.001
Lysyl hydroxylase	8.2 ± 3.5	151 ± 37	<0.001
Hydroxylysyl galactosyltransferase	31.8 ± 10.8	42.2 ± 12.7	NS
Galactosylhydroxylysyl glucosyltransferase	298 ± 32	530 ± 44	<0.001

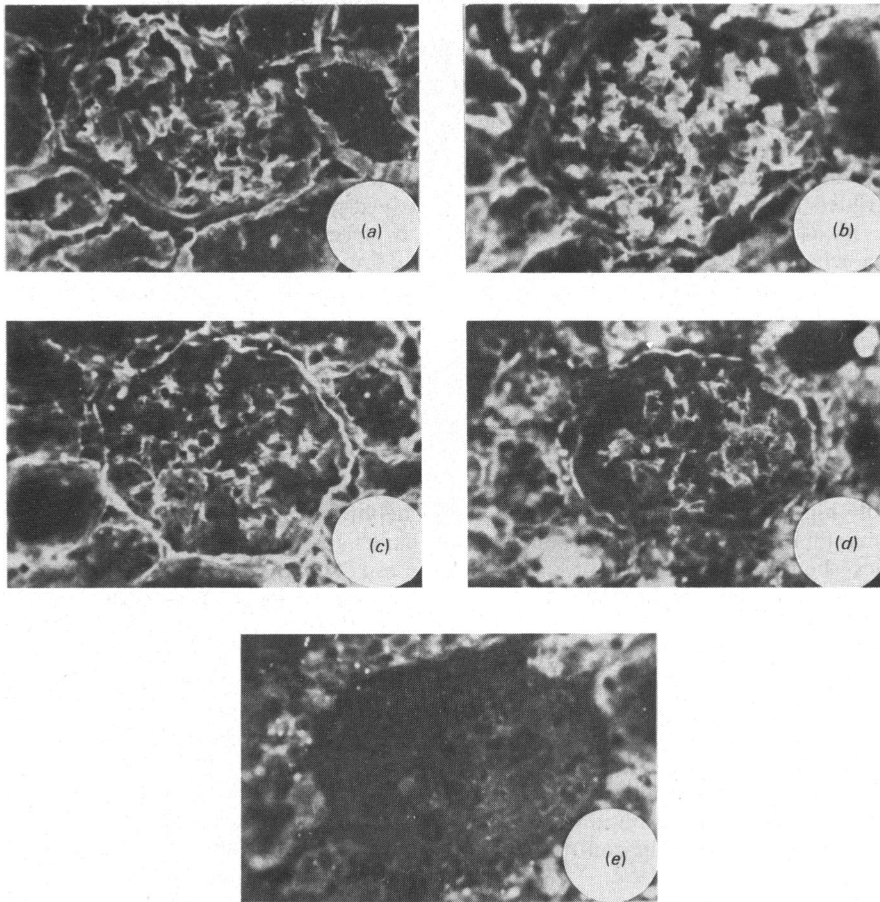

Fig. 2. *Indirect immunofluorescence localization of basement-membrane proteins in glomeruli at day 9 after administration of PAN*

The sections were stained with anti-(human laminin P1) antibodies (*a* and *b*), with anti-(human 7S collagen) antibodies (*c* and *d*), or with non-immune rabbit serum (*e*). There is autofluorescence in the epithelium of the proximal tubules in each case. Controls are shown in (*a*) and (*c*), and samples from nephrotic rats in (*b*), (*d*) and (*e*). Magnification ×160.

larger in size than the P2 fragment produced *in vitro* (Fig. 4*a*). In the nephrotic-rat sera, it was the first peak in particular that was increased (Fig. 4*b*). Both of the larger antigen forms reacted in the radioimmunoassay for the P1 domain of laminin (results not shown), indicating that the forms of laminin present in serum contained both the P1 and the P2 regions of the molecule.

Discussion

It has been reported that the staining of GBM with antibodies against the basement-membrane-specific heparan sulphate proteoglycan is diminished in PAN-induced nephrosis, but there are no apparent changes in the stainings for laminin or type IV collagen (Mynderse *et al.*, 1983). Another paper described the aggravation of proteinuria in PAN nephrosis by exogenous anti-laminin antibody (Abrahamson *et al.*, 1983). An earlier study demonstrating a decrease in collagen-specific amino acids in the GBM (Kefalides & Forsell-Knott, 1970) suggested an involvement of the collagen of the GBM in this condition. Thus the present study was undertaken with two objectives: firstly, to elucidate the possible changes in the two basement-membrane proteins laminin and type IV collagen by quantitative methods, and secondly, to find out whether PAN nephrosis is accompanied by changes in some enzyme activities needed for collagen synthesis in kidney.

We find an increase in the serum concentration of laminin-related antigens in acute PAN nephrosis. This change is specific, in that no such altera-

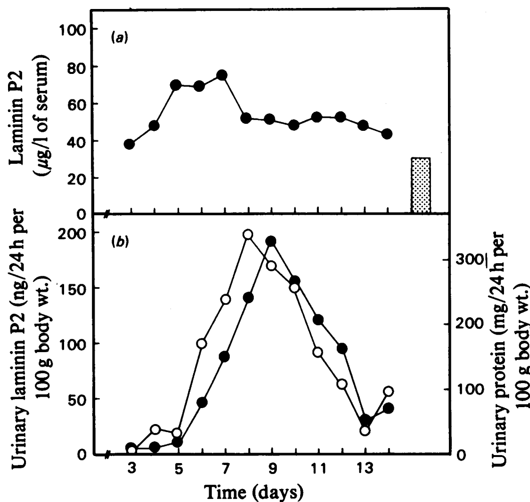

Fig. 3. *Serum and urinary laminin P2 antigen in nephrotic rats as a function of time*

(*a*) ●, Serum concentration of laminin P2 antigen. The points represent the means for two animals. The column indicates the mean concentration in control animals. (*b*) ●, Urinary excretion of laminin P2 antigen; ○, urinary excretion of protein. The points represent the means for six animals.

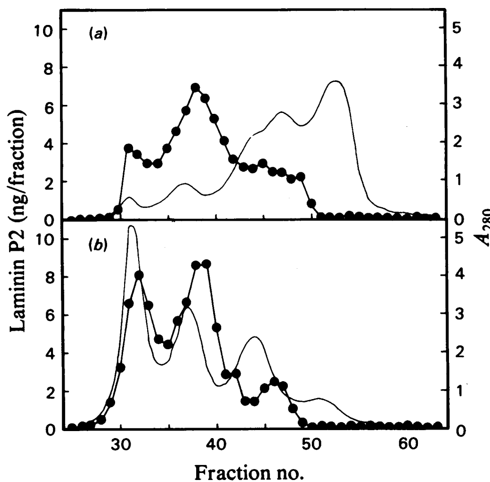

Fig. 4. *Assessment of the molecular size of the laminin P2 antigen in serum by gel filtration on a Sephacryl S-300 column*

Protein absorbance (——) was measured at 280 nm. Laminin P2 antigen (●) in the fractions was measured by radioimmunoassay. (*a*) Control serum; (*b*) serum from a nephrotic rat at day 9.

tion is seen in type-IV-collagen-derived fragments. It would be interesting to know whether similar changes could be demonstrated for the heparan sulphate proteoglycan. Although this basement-membrane component seems to be nearly absent in the GBM in fully developed PAN nephrosis, no time-course for this alteration has been reported. It has not been demonstrated that the increased serum laminin originates from the GBM, but at present such an assumption seems feasible. Relatively little is known about the half-lives of the various basement-membrane components. Assuming that PAN prevents the deposition of new basement-membrane material by the glomerular epithelial cells, the effect should first be visible in those components whose half-lives are the shortest, i.e. in heparan sulphate, laminin and other non-collagenous components.

We found no difference in the staining of the nephrotic-rat glomeruli for type IV collagen or laminin, compared with control kidneys. This supports previous findings (Abrahamson *et al.*, 1983; Mynderse *et al.*, 1983). Also the quantitative measurements of these proteins in kidney-cortex tissue of PAN-treated rats failed to demonstrate specific decreases in laminin. However, the large amount of basement-membrane material present in the tubuli could easily conceal a slight decrease in the laminin content of the glomeruli. For study of this possibility, isolated glomeruli should be analysed.

Although we found no changes related to type IV collagen in the serum or urine or at the tissue level, a surprisingly large stimulation of three intracellular enzyme activities of collagen biosynthesis took place in the kidney. The pattern observed, prolyl 4-hydroxylase and lysyl hydroxylase changing most and the two sugar transferases only slightly, however, resembled that observed in liver injury (Risteli & Kivirikko, 1976) and experimental diabetes (Risteli *et al.*, 1976). It is probably related to increased synthesis of interstitial collagens after tissue damage rather than to an elevated basement-membrane collagen production. Indeed, repeated administration of PAN has been shown to induce focal sclerosis of glomeruli (Glasser *et al.*, 1977), where such a fibrotic change is likely. The increases in the hydroxylase activities were remarkably high. The 18-fold increase in lysyl hydroxylase activity seems reliable, because changes of the same magnitude were found with several batches of protocollagen substrates, by using both [^{14}C]- and [^{3}H]-lysine label, and because the chemical assay of the labelled hydroxylysine formed is highly specific (Kivirikko & Myllylä, 1982).

In normal fibroblasts PAN inhibits the synthesis of RNA and arrests the cells in the G_1 phase of the cell cycle (Studzinski & Gierthy, 1973). However, it is not known whether the mechanism is similar in glomerular epithelial and endothelial cells. It also remains to be studied whether the enzymes of

collagen biosynthesis are activated in kidney fibroblasts independently of RNA synthesis or rather as a reparatory response, when the lesion is subsiding.

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