Proteinuria in Pigs
with Experimentally Induced Renal Damage ¹

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There is substantial evidence for the statement that the manner of elimination and excretion of proteins by the kidney is not uniform but depends on the characteristics of the specific proteins, mainly on molecular size and shape, but probably also on other properties (2, 3, 11, 14-16). Consequently knowledge on this subject can be obtained only by analysis of specific proteins. Such studies have been performed earlier mainly by analysis of specific proteins in the plasma and urine of patients with different renal diseases, and experimentally by studying the fate of heterologous proteins injected into laboratory animals.

The results of animal experiments have added much knowledge but the conclusions may be hazardous since the kidneys may handle homologous and heterologous proteins in different ways. No studies have hitherto been published concerning the urinary excretion of homologous proteins in laboratory animals with renal damage. The value of an experimental model in the study of proteinuria is obvious; we therefore decided to investigate the renal handling of homologous proteins in the pig.

Quantitative analysis of specific proteins most often demands their isolation in order to raise antibodies. We were initially interested in the renal handling of low molecular weight (LMW) proteins. Such proteins are usually isolated from the urine of patients with proximal tubular damage (1, 10) or chronic renal failure (5), but considerable difficulties arose in our attempts to induce LMW proteinuria by tubulotoxic substances, raising new questions about the renal handling of such proteins.

In this preliminary report results are presented on porcine proteinuria in different types of renal damage.

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Materials and Methods

Experimental design. 13-15 kg male pigs of Swedish land race were used. In intravenous

ketamine (Ketalar®)-droperidol (Dridol®) anaesthesia an indwelling polyethylene catheter was placed into the internal jugular vein. Cystostomy was performed with a permanent catheter and the urethra was ligated and divided to ensure complete urine sampling. The bladder catheter was connected to a sterile plastic bag placed outside the cage. During the experiments the animals were kept in individual small cages at room temperature. They were fed every day with 1 kg of a fodder mixture containing the essential vitamins, trace metals, 14.5-percent digestible protein, and 2.65 Mcal/kg. They were allowed free access to water.

In one experiment two pigs were made uremic by 5/6 nephrectomy (Ne1 and Ne2). This procedure was performed transabdominally in connection with the above-mentioned operation by resection of the upper and lower pole of one kidney followed by contralateral nephrectomy.

In another experiment two pigs were injected by 10-percent potassium dichromate dissolved in 0.9-percent saline into the jugular vein via the indwelling catheter. One pig (Di1) received increasing doses at intervals of 3 days, starting with 0.5 mg/kg body weight and amounting totally to 112 mg. The other pig (Di2) received 100 mg in a single injection.

In a third experiment three pigs were injected by a 10-percent solution of maleic acid adjusted to pH 7.4 by 1 M NaOH. One pig (Ma1) received 360 mg/kg body weight in one injection, one pig (Ma2) received on day one 240 mg/kg body weight and on day four an injection of 360 mg/kg body weight, and one pig (Ma3) received daily injections of increasing doses of maleic acid: the initial dose was 150 mg/kg body weight and the total dose in the course of 16 days was 38 g.

Two further pigs were operated and used as controls.

Injections were started after a control period of varying length. Values obtained from these periods were also used as controls. Urine was collected, as a rule, every 24 h and 8-10 ml EDTA blood was drawn every 24 h when possible. Shorter intervals were used immediately after the injections of maleic acid. Urine was also drawn from the urinary bladder with a syringe immediately before the cystostomy. These samples were used as preoperative controls.

Analytical methods. Each 24-hour urine sample was filtered and analyzed for total protein, glucose, phosphorus, sodium, creatinine, and for its content of a porcine LMW (α -PLMW) protein. This protein was previously isolated and characterized by us as a LMW protein (13) with a molecular weight of about 12,000.

Each 24-hour urine sample was concentrated by ultrafiltration against solid polyethylene glycol (MW 20,000) with Visking dialyzing tubing of 23/32-inch inflated diameter.

Agarose gel electrophoresis was performed on the concentrated urine and 38 selected samples were subjected to gel chromatography.

The Q-PLMW protein was analyzed by single radial immunodiffusion with a modification (12) of the procedure described by Mancini et al. (8). Antiserum against the α -PLMW protein was raised in rabbits and dilutions of the isolated protein were used as standard.

Sodium and potassium were determined by flame photometry, phosphorus according to Itaya and Vi (4), and total protein by a biuret method. Glucose was determined semiquanti-

tatively by the use of Labstix®. Creatinine was analyzed in a Technicon autoanalyzer (2). Agarose gel electrophoresis was performed according to Johansson (5). Gel chromatography was carried out on a 60 X 0.9 cm column containing Sephadex G-75 superfine equilibrated with 0.01 M Tris-HCl buffer, pH 7.4, containing 0.2 M NaCl and 0.05-percent sodium azide. Fractions of 2.8 ml were collected at a flow rate of 5.6 ml/h.

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Renal tissue, obtained by needle biopsy or autopsy at the end of each experiment, except after 5/6 nephrectomy, was studied by light microscopy.

Calculations. Fractional excretion of sodium and phosphorus, and the or-PLMW protein/creatinine clearance ratio were calculated according to the formula:

where U_x and P_x represent the concentration of the substance x in urine and plasma, and U_{Cr} and P_{Cr} that of creatinine.

Results

Normal Values

The mean normal plasma level, the urinary excretion, and the protein/creatinine clearance data are given in table I. As seen in the table an increase of the plasma level was observed following the surgical procedures. As a rule, this elevation of the plasma level persisted only one or two days. An increase of the

Table I. Control values observed and calculated from plasma and bladder urine obtained during the operation and from the postoperative period before the induction of renal damage

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Table II. Pertinent data from observations made before and after the induction of renal damage

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urinary level was also observed but the calculated -PLMW protein/creatinine clearance ratio was unchanged.

Pigs Intoxicated with Potassium Dichromate

Injection of potassium dichromate did not induce glucosuria or an increased phosphaturia. Creatinine clearance decreased slightly during the latter part of both experiments. Total protein excretion increased slightly in both experiments. After two doses of dichromate the excretion of a-PLMW protein in Di 1

increased to a maximal amount of 80 mg/24 h but as the serum level also increased considerably, the C-PLMW/CCr ratio remained normal. After the last dose of 37.5 mg the pig became anuric and died 3 days later. A kidney biopsy specimen taken before the last dose showed extensive tubular damage in combination with wide-spread interstitial inflammation and oedema.

Fig. 1. Course of a pig (Ma2) injected (dotted lines) with 240 mg/kg maleic acid on day 1 and with 360 mg/kg on day

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Fig. 2. Correlation between fractional phosphate excretion and the -PLMW/creatinine clearance ratio in control pigs (•) and in pigs intoxicated with maleic acid (○).

Fig. 3. Correlation between creatinine clearance and the -PLMW/creatinine clearance ratio in control pigs (•), in 5/6 nephrectomized pigs (○) and in pigs intoxicated with maleic acid (○).

Pigs Intoxicated with Maleic Acid

Intravenous injection of maleic acid resulted in an immediate increase of urinary flow (470 ml/h in Mal, 240 ml/h in Ma2), whereas no increase was seen in Ma3, which was given the smallest initial dose. In the course of about 12 h Mal became anuric and remained so until death 3 days later. During a similar period of time the urine output of Ma2 became normal but the glomerular filtration rate decreased to about 5-10 % of the initial level (fig. 1). The increased diuresis was followed by a heavily increased natriuresis which reached an excretion of 20 % of the filtered load, by phosphaturia amounting to values between 40 and 100 % of the filtered load, and by glucosuria. In Ma2 the increased sodium and phosphate excretion almost normalized after 3-4 days and the glucosuria disappeared. The injections were also followed by a rise in (-PLMW excretion. No correlation was found, however, between (-PLMW and phosphate excretion (fig. 2), whereas an inverse correlation was observed between (-PLMW excretion and the clearance of creatinine (fig. 3).

5/6 Nephrectomized Pigs

An immediate decrease in the glomerular filtration rate to levels of about 10- 20 % of the control values followed 5/6 nephrectomy in Ne 1 and Ne2.

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Fig. 4. Gel chromatography on Sephadex G-75 superfine of selected samples of concentrated urine. The UV absorption of each fraction (points) and their content of -PLMW protein (triangles) is indicated. As control, pooled urine was used from six pigs, the urine

being taken from the postoperative periods before renal damage was induced. Creatinine and α -PLMW clearance data, and the plasma concentration of α -PLMW protein observed simultaneously are given. The control values are the mean values for the six animals. The same ratio between UV absorption and α -PLMW protein concentration is used in each graph. The letter A denotes the albumin peak.

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Simultaneously an increase of α -PLMW and total proteinuria was observed. The calculated C-PLMW/CCr ratio on several days exceeded 1 in Ne2, whereas in Ne1 it fluctuated between 0.2 and 1. During the following days, total protein excretion increased further, whereas the α -PLMW excretion remained constant (Ne2) or decreased slightly (Ne1).

Gel Chromatography

Separation in Sephadex G-75 showed that the main bulk of the proteins in the urine from the postoperative controls consisted of albumin and proteins with a similar or larger molecular size.

In the urine from the pigs intoxicated with maleic acid there was an even higher proportion of proteins with a greater molecular size both in the early course and after the development of chronic renal failure. In the pigs intoxicate

Fig. 5. Agarose gel electrophoresis of the pooled LMW protein fractions from gel chromatography of concentrated porcine urine, of selected samples of concentrated urine from pigs with induced renal damage, of concentrated urine from the postoperative period before the induction of renal damage, and of porcine plasma. The arrows indicate the α -PLMW protein.

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with dichromate most of the proteins were eluted in the albumin peak. Immediately after 5/6 nephrectomy, a large part of the protein consisted of proteins with a molecular size less than that of albumin but the proportion of LMW protein diminished rapidly with time, indicating that the simultaneous increase of total protein excretion was caused by high molecular weight proteins. After days 3- 4 the elution pattern became strikingly similar to that of control urine (fig. 4).

Agarose Gel Electrophoresis

The patterns revealed by agarose gel electrophoresis of the concentrated urine samples were compared with those produced by porcine plasma and by the pooled LMW fractions eluted by gel chromatography.

The only situation in which a LMW protein ('tubular') pattern was observed, was on days 2 and 3 after 5/6 nephrectomy.

Faint bands with the same position as the cathodal LMW proteins were visible after maleic acid intoxication and later than day 3 after 5/6 nephrectomy. Faint bands with the same position as the α -PLMW protein were recognizable after potassium dichromate intoxication, but the total patterns mainly resembled the plasma pattern (fig. 5).

Discussion

The main purpose of the present pilot study was to find a way of producing a sufficient LMW protein excretion to allow an easy preparation of suitable proteins. Consequently the approach varied in each experiment and since the number of pigs in each group was small, usual statistical calculations were not possible. For these reasons any conclusions should be drawn with great care. There is sufficient evidence to state, however, that the urinary excretion of the α -PLMW protein studied in the undamaged pigs exceeded by far the excretion of any of the LMW proteins in healthy humans. Thus, the mean C-PLMW/CCr ratio of 5.7×10^2 exceeded the same ratio of human-micro globulin (6) by several hundred times. With a mean concentration of 21 mg/l α -PLMW protein in normal porcine urine, the isolation of this, and possibly also other LMW proteins, from such material may be feasible. Because of the relatively small amount of high molecular weight protein, the urine produced in the first days after 5/6 nephrectomy is undoubtedly the most suitable for preparative purposes; and such material was also used in the first preparation of the α -PLMW protein.

The cause of the increased LMW proteinuria in the 5/6 nephrectomized pig is uncertain. An increased LMW proteinuria is usual in all causes of renal failure

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in man (6). The suggested mechanism behind this phenomenon is a combination of an increased load (elevated plasma level and increased tubular flow) and proximal tubular dysfunction caused by the uremic intoxication. Except for an increased tubular flow, these explanations cannot be valid for the α -PLMW protein, since the increased excretion appeared immediately after the operation and the plasma levels were only moderately elevated. Neither can the surgical trauma be rendered responsible since no increase was observed after operations of almost similar magnitude performed on the pigs in the other groups. As the Ca-PLMW/CCr ratio in several cases exceeded 1, indicating a urinary clearance of α -PLMW protein greater than the clearance of creatinine, the possibility of tubular secretion of α -PLMW protein should be considered. The present observations

are not sufficient to confirm such a mechanism since the changes of the plasma levels of creatinine and a-PLMW protein were rapid, invalidating the accuracy of the clearance calculations. Another explanation may be, that the a-PLMW creatinine clearance ratio does not reflect percentage a-PLMW filtration since creatinine clearance underestimates the glomerular filtration rate in the pig by 10-15 %⁽⁹⁾.

An increased tubular flow causing an insufficient tubular reabsorption of a-PLMW protein is also doubtful since the experiments with the tubulotoxic substances cast doubt on the usual concept of renal handling of LMW proteins, at least in the pig. Judged both from the chromatographic studies, from agarose gel electrophoresis, and from determination of the a-PLMW protein the increased proteinuria in the pigs intoxicated with maleic acid and presenting laboratory and microscopic evidence of proximal tubular damage, was dominated by the high molecular weight species. An increased LMW proteinuria was seen only when the glomerular filtration rate was considerably decreased. In Di2 the excretion of a-PLMW protein was increased but the calculated Ca-PLMW/CCr ratio was normal in spite of microscopic evidence of tubular damage and of a heavily increased tubular load caused by the high plasma level.

The results from other studies also suggest that the a-PLMW protein is not filtered by the glomerulus and reabsorbed by the proximal tubular cells, the mechanism suggested for the human LMW proteins. In an experimental study of fungal nephritis caused by Ochratoxin-A in pigs (7) the excretion of the a-PLMW protein was also studied. In pigs with proximal tubular damage, reflected by a substantial decrease of TmPAH and TmPAH/CIn, by the presence of glucosuria, and by microscopical investigation of the kidneys, no significant increase of a-PLMW protein excretion and clearance was observed.

Although other mechanisms of protein handling may dominate in man, a more thorough understanding of these mechanisms may be elucidated by studying the pathways in other species. No definite explanation of the present findings, in many ways diverging from the current concepts of proteinuria, are at hand, but we are convinced that analysis of homologous protein excretion in

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experimental renal damage may be useful in the study of renal handling of protein. Several protein species should be studied, preferably analogues to the human proteins.

Summary

Proteinuria was studied experimentally in pigs with renal damage induced by maleic acid, potassium dichromate and by 5/6 nephrectomy. The methods used were: analysis of

total protein, immunochemical quantitation of a porcine low molecular weight (-PLMW) protein, agarose gel electrophoresis, and gel chromatography of concentrated urine samples. The urinary clearance of the -PLMW protein in normal pigs was considerably higher than LMW protein clearance in normal man. No increase was seen after surgery. The induction of proximal tubular damage resulted in an increased excretion of, above all, high molecular weight proteins (;;albumin) but also of low molecular weight proteins. The highest increase in -PLMW clearance was seen immediately after 5/6 nephrectomy, and the increased -PLMW excretion in pigs with proximal tubular dysfunction was better correlated to creatinine clearance than to the degree of phosphaturia or glucosuria. The results suggest different renal handling of plasma proteins in pigs and humans.

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