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# Enalapril and low protein reverse chronic puromycin aminonucleoside nephropathy

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**Enalapril and low protein reverse chronic puromycin aminonucleoside nephropathy.** The effects of dietary protein and converting enzyme inhibition (CEI) on chronic puromycin aminonucleoside nephropathy (PAN) were studied. PAN was induced with seven SQ injections of puromycin aminonucleoside 20 mg/kg over 10 weeks in male Sprague-Dawley rats. The rats were divided into a 22.5% protein diet group (Gr 1), a 6% protein diet group (Gr 2), and an enalapril-treated group on 22.5% protein diet (Gr 3). Group 4 animals served as age-matched controls. Both diets were isocaloric and had the same phosphorus content. Rats from groups 1, 2, and 4 were sacrificed at 12, 18 and 24 weeks. Five rats of group 3 were sacrificed at 12 weeks, and the others were divided in subgroups 3A (diet changed to 6% protein) and 3B (no changes); half of each subgroup was sacrificed at 18 and 24 weeks, respectively. Group 2 had significantly less proteinuria than group 1 at all times. Group 3 had the same proteinuria as group 1 until 12 weeks and then began to decrease. In group 3A proteinuria decreased to group 2 levels, while in group 3B the decrease was slower but still prominent. Early lesions of focal and segmental glomerular sclerosis/hyalinosis (FSH) were present in groups 1, 2, 3 at 12 weeks (16 ± 1.2%, 15 ± 1.3%, 7 ± 1.3%, respectively, versus 1.3 ± 0.4% in controls), but by 18 weeks a reversal in FSH was seen in groups 2 and 3A/B (3 ± 1.6%, 2 ± 0.4%, and 3 ± 0.9%, respectively, vs. 14 ± 1.5% in group 1). This reversal persisted at 24 weeks (5 ± 2.5%, 3 ± 0.8%, 4 ± 0.8% vs. 18 ± 2.6%). At 24 weeks mean glomerular diameter was significantly less in group 2 compared to group 1, 100.7 ± 2.0 $\mu$ versus 112.2 ± 2.7 $\mu$, $P = 0.009$. In summary, both low protein diet and CEI for 24 weeks reversed both proteinuria and early FSH lesions in chronic PAN after cessation of PA injections.

Focal and segmental glomerular sclerosis/hyalinosis (FSH) can be the cause of idiopathic nephrotic syndrome and is the principal lesion in heroin-induced nephropathy, reflux nephropathy, and the final stages of other glomerulopathies [1].

In the rat FSH develops spontaneously with aging [2], after renal ablation [3, 4], with induction of diabetes mellitus [5], after administration of adriamycin [6, 7] and in puromycin aminonucleoside nephropathy [8–10].

The pathogenesis of FSH in humans and in animal models is not known. According to one hypothesis, FSH is due to mesangial cell proliferation and matrix expansion following various stimuli to mesangial cells [4, 11–13]. An alternative hypothesis holds that FSH is the consequence of toxic or other damage to visceral epithelial cells with subsequent vacuolation, degeneration and detachment of these cells from the basement membrane eventually leading to collapse of capillaries accompanied by development of "hyaline" insudative lesions in the glomerular capillary lumina [14, 15]. In this scheme epithelial lesions are therefore considered as an early stage of segmental sclerosis.

In a previous study [16], we induced chronic puromycin aminonucleoside nephropathy (PAN) and examined the effects of angiotensin converting enzyme inhibition (CEI) on its course. We found that 12 weeks of CEI had no effect on the degree of proteinuria or histologic alterations observed in this model. We now wished to test whether continuing treatment with a converting enzyme inhibitor after cessation of aminonucleoside injections would result in any improvement in the course of this model. We also tested the effects of low protein diet either for the duration of the experiment or after completion of the injection schedule with or without concomitant CEI.

## Methods

### *Animals*

Adult male Sprague-Dawley rats weighing 160 to 200 g at the beginning of the experiment were used.

### *Chemicals*

Puromycin aminonucleoside ($N^6$, $N^6$-dimethyl-[3-amino-3-deoxy] adenosine) was purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). Enalapril ([S]L-[N-(L-ethoxycarbonyl-3-phenylpropyl)-l-alanyl]-L-proline) in powder form was obtained from Merck Sharp and Dohme (West Point, Pennsylvania, USA).

### *Food*

Both kinds of food (22.5% and 6% protein) were purchased from Teklad (Madison, Wisconsin, USA). The protein used was casein and both diets were isocaloric (3.55 Kcal/g). They had the same amount of fat (5.5%), calcium (0.97%), phosphorus (0.85%) and magnesium (0.21%). The 22.5% diet had 51.6% carbohydrates and 6% cellulose and the 6% diet had 73.2% carbohydrates and 8.2% cellulose.

### *Biochemistry*

Urine protein determinations were performed by the sulfosalicylic acid method. Serum creatinine was measured by the Jaffe

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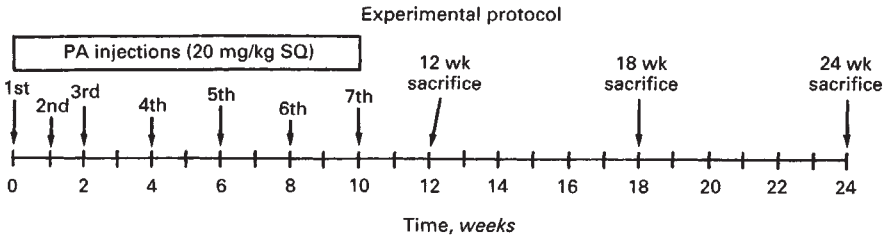


**Fig. 1.** *Experimental protocol for the induction of chronic puromycin aminonucleoside nephropathy.* Puromycin was injected weekly for three weeks and then biweekly for eight more weeks. Animals were sacrificed at 12 weeks, 18 weeks or 24 weeks.

**Table 1.** Experimental groups

| | |
|---|---|
| Group 1: | Rats with chronic PAN on a 22.5% protein diet ($N$ = 24) |
| Group 2: | Rats with chronic PAN on a 6% protein diet ($N$ = 24) |
| Group 3: | Rats with chronic PAN on enalapril and 22.5% protein diet ($N$ = 31) |
| | After 12th week: Group 3A: Diet changed to 6% protein |
| | Group 3B: Continued as before |
| Group 4: | Age-matched controls on a 22.5% protein diet ($N$ = 12) |

method. Serum protein was measured by the Lowry method. Serum cholesterol (fasting) was measured by the enzymatic method [17] in a Hitachi 737 multichannel chemistry analyzer.

### *Blood pressure measurements*

Systolic blood pressures were measured indirectly with a tail-cuff sphygmomanometer (NARCO Biosystems, Houston, Texas, USA) connected to a recorder.

### *Protocol*

The experimental protocol is shown in Figure 1. The rats were initially divided into four groups (Table 1). Baseline studies included blood pressure (BP) measurements, 24-hour urine collection for protein, and serum creatinine and protein determinations.

Throughout the experiment the urine protein was measured monthly and before sacrifice. Serum creatinine and protein were measured every two months and before sacrifice. Serum cholesterol was measured before the third sacrifice in each group. BP was determined before each sacrifice.

Group 1 animals ($N$ = 24) received seven subcutaneous injections of puromycin aminonucleoside (PA; 20 mg/kg diluted in normal saline, concentration 8 to 9 mg/ml). The first three injections were given at weekly intervals, and the last four at biweekly intervals [9]. These animals had free access to water and were given a 22.5% protein diet. Group 2 rats ($N$ = 24) received PA as described above, had free access to water and were given a 6% protein diet. Group 3 rats ($N$ = 31) received PA as above and were given a 22.5% diet and enalapril 5 to 10 mg/kg/day [16, 18]. These were housed in individual cages and enalapril was dissolved in drinking water. The solution was replaced every 48 hours, and its daily consumption was calculated. Group 4 rats ($N$ = 12) were used as age-matched controls; they were on 22.5% protein diet.

One-third of the rats of groups 1, 2 and 4 were sacrificed at 12, 18 and 24 weeks. Group 3 had five rats sacrificed at week 12 and the remaining animals were randomized on the basis of their proteinuria into two subgroups. Subgroup 3A ($N$ = 13) was given the 6% diet while continuing on enalapril. Subgroup 3B ($N$ = 13) was given the 22.5% diet and enalapril. One-half of each subgroup were sacrificed at 18 and 24 weeks.

At the times of sacrifice, the animals were fasted overnight, anesthetized with ether, blood samples were drawn, and immediately afterwards the kidneys were removed, weighed and each of them processed separately.

### *Tissue processing*

One-half of each kidney was fixed in alcoholic Bouin's solution, embedded in methacrylate, sectioned at 2 $\mu$m thickness and stained with periodic acid-Schiff reagent. Three sections, each 200 $\mu$m from one another to eliminate assessing the same glomerulus more than once, were prepared from each kidney.

Tissue for immunofluorescence was processed by immersion in isopentane and snap-freezing in liquid nitrogen. Four $\mu$m sections were cut. These were stained directly with fluorescein isothiocyanate (FITC) antibodies raised in goat against rat albumin, fibrinogen, IgM, IgG and C3 (Cooper Biomedical, Inc., Malvern, Pennsylvania, USA). Before use the purity, specificity and concentration of the FITC antibodies were checked by Ouchterlony double immunodiffusion [19] and by immunoelectrophoresis.

A small portion of each kidney was divided into 1 to 2 mm cubes, fixed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, and embedded in Epon. One-micron-thick sections, stained with toluidine blue, were examined by light microscopy; thin sections stained with uranyl acetate and lead citrate were examined with an electron microscope [14].

### *Morphological assessment*

The glomeruli were quantitated for percentage involved with segmental sclerosis, and the stage or stages of evolution of this lesion was semiquantitively tabulated. Glomerular morphometry was performed using standard methods [20]. By moving the slide from subcapsular to juxtamedullary zones, one hundred consecutive glomeruli were measured with an eyepiece micrometer. Maximal capillary tuft diameters for each glomuerulus were measured. Each kidney was evaluated without the observer's knowledge of the animal group to which it belonged.

### *Statistics*

The unpaired Student's $t$-test was used for proteinuria, serum creatinine, serum protein, and serum cholesterol and BP measurements [21].

The Mann-Whitney U-test for nonparametric values was used for histologic data [21]. The histological score of each kidney was considered individually for this calculation. Differ-

**Table 2.** Body weight (grams)

| | Group 1 | Group 2 | Group 3 | | | Group 4 |
|---|---|---|---|---|---|---|
| | | | 3A | | 3B | |
| 8 weeks | 367 ± 21 | 256 ± 27[a] | | 350 ± 19[a,b] | | 389 ± 38 |
| 12 weeks | 375 ± 28 | 220 ± 16[a] | | 322 ± 22[a,b] | | 407 ± 35 |
| 18 weeks | 420 ± 53 | 275 ± 20[a] | 352 ± 36[a,b] | | 355 ± 47[a,b] | 422 ± 27 |
| 24 weeks | 421 ± 16 | 317 ± 50[a] | 339 ± 24[a,b] | | 400 ± 17[a,b] | 473 ± 28 |

[a] $P < 0.05$ group 1 versus group 2, group 3, group 3A, group 3B
[b] $P < 0.05$ group 2 versus group 3 or 3A and 3B

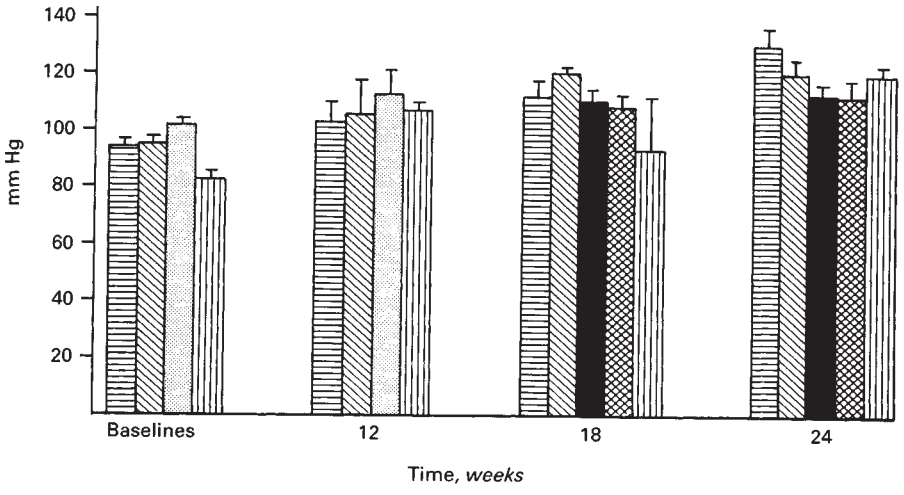


**Fig. 2.** *Systolic blood pressure in all five groups of animals.* Only group 1 at 24 weeks was hypertensive (131 ± 7 mm Hg). Symbols are: (▤) group 1; (▧) group 2; (▨) group 3; (■) group 3A; (▩) group 3B; (▥) group 4.

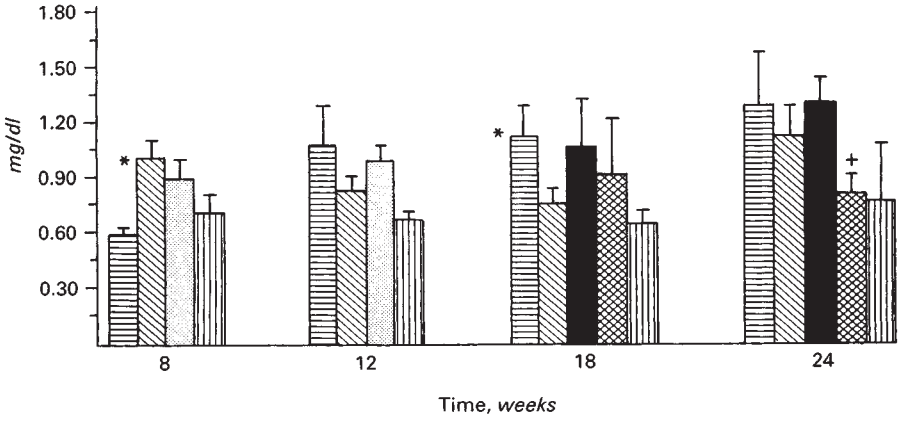


**Fig. 3.** *Serum creatinine in mg/dl.* $*P < 0.05$ for group 1 versus group 2, + $P < 0.05$ for group 1 versus group 3B. Symbols are: (▤) group 1; (▧) group 2; (▨) group 3; (■) group 3A; (▩) group 3B; (▥) group 4.

ences were considered significant if the $P$ value was less than 0.05. Results are expressed as mean ± SEM.

## Results

### *Animals*

Group 1 and group 3 animals had slightly decreased growth compared to the controls (Table 2). However, growth was profoundly stunted in group 2 compared to all other groups (Table 2).

### *Blood pressures*

A longitudinal trend towards higher systolic blood pressure was seen in all groups, most prominently in group 1 (Fig. 2). No consistent intergroup differences were observed at individual points of time, and the animals remained normotensive (except group 1 at 24 weeks, 131 ± 7 mm Hg).

### *Serum creatinine*

There were no consistent patterns in serum creatinines (Fig. 3). Overall, group 1 animals tended to have higher average values than group 2 or group 3, 1.5 ± 0.2 mg/dl (Gr 1), 1.1 ± 0.2 (Gr 2), 1.3 ± 0.1 (Gr 3A), 0.8 ± 0.1 (Gr 3B) versus 0.8 ± 0.2 of controls at 24 weeks.

### *Serum protein*

In the early part of the study group 2 animals had lower levels of total serum protein than those of group 1, but this difference

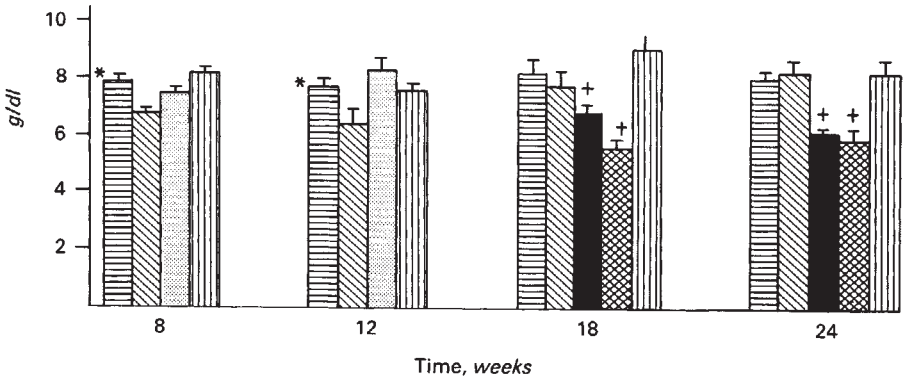


**Fig. 4.** *Serum protein in g/dl.* $*P < 0.05$ for group 1 versus group 2, $+ P < 0.05$ for group 1 versus group 3, 3A or 3B. Symbols are: (▤) group 1; (▧) group 2; (▨) group 3; (■) group 3A; (▩) group 3B; (▥) group 4.

**Table 3.** Proteinuria mg/24 hr

| | | | Group 3 | | |
|---|---|---|---|---|---|
| | Group 1 | Group 2 | Group 3A | Group 3B | Group 4 |
| 4 weeks | 125 ± 11[a] | 75 ± 10 | 114 ± 17 | | 10 ± 2 |
| 8 weeks | 292 ± 33[a] | 151 ± 20 | 265 ± 26[b] | | 17 ± 2 |
| 12 weeks | 299 ± 37[a] | 135 ± 15 | 320 ± 26[b] | | 16 ± 2 |
| | | | 348 ± 45 | 309 ± 36 | |
| 15 weeks | | | 60 ± 8 | 126 ± 28[c] | |
| 18 weeks | 240 ± 35[a] | 20 ± 4 | 70 ± 5[d,e] | 90 ± 14[d,e] | 14 ± 3 |
| 21 weeks | 278 ± 44[a] | 17 ± 7 | 32 ± 6[d] | 55 ± 14[d,e] | 19 ± 3 |
| 24 weeks | 209 ± 29[a] | 25 ± 8 | 27 ± 11[d] | 53 ± 14[d] | 24 ± 3 |

[a] $P < 0.05$ between 1 and 2
[b] $P < 0.05$ between 3 and 2
[c] $P < 0.05$ between 3A and 3B
[d] $P < 0.05$ between 1 and 3A/3B
[e] $P < 0.05$ between 2 and 3A/3B

disappeared in the second half of the study (Fig. 4). The rats on enalapril (with or without superimposed low protein diet) showed a significant decrease of total serum protein in comparison to all other groups at the second half of the experiment. The rats did not become hypoproteinemic (<6.0 g/dl) at any point, except group 3B at 18 weeks (5.5 ± 0.3 g/dl). Total protein was measured rather than albumin since the proteinuria in this model is non-selective except at the beginning [22].

### *Fasting serum cholesterol*

Serum cholesterol levels at the end of the experiment (week 24) were significantly higher in group 1 (286 ± 20 mg/dl) compared to group 2 (148 ± 24 mg/dl), group 3A (119 ± 7 mg/dl), groups 3B (107 ± 8 mg/dl) and group 4 (101 ± 2 mg/dl), $P < 0.05$. There were no significant differences between groups 2, 3A, 3B and controls.

### *Proteinuria*

All animals in the experimental groups became heavily proteinuric with a progressive increase of proteinuria until the 12th week (Table 3). Until that time the proteinuria in groups 1 and 3 evolved similarily. Group 2 rats, while significantly proteinuric compared to controls, were consistently below groups 1 and 3 ($P < 0.05$), especially at eight weeks and later. After 12 weeks and until the end of the experiment, there was a decrease in protein excretion in group 2, down to control levels. In contrast, group 1 rats remained heavily proteinuric throughout the experiment. Group 3A also showed improvement in proteinuria and by the 21st week these animals were similar to the controls. Groups 3B had a slower decrease in proteinuria, but by the 24th week they were not different from controls.

### *Pathology*

On gross examination, kidneys in group 1 were enlarged and pale with a granular surface. In group 2, the same appearance was seen at 12 weeks but at 18 and 24 weeks the kidneys were not as enlarged or granular. Group 3 had similar appearance to groups 1 and 2 at 12 weeks. Group 3A kidneys had a gradual decrease in size and granularity from 18 to 24 weeks, while group 3B had a lesser degree of improvement.

The weights of the kidneys were averaged and divided by body weight to account for variations of growth between groups (Fig. 5). These ratios were significantly higher for groups 1, 2, and 3 compared to controls at 12 weeks. At the 18th week and 24th week sacrifices, there was a significant decrease of this ratio to control levels in group 2. There was a similar decrease in group 3A. In group 3B the ratio remained elevated until 24 weeks.

Measurement of glomerular morphometry at 24 weeks demonstrate that Group 2 had a significantly smaller mean glomerular diameter than Group 1, 100.7 ± 2.0 $\mu$ versus 112.2 ± 2.7 $\mu$, $P = 0.009$. At 24 weeks Group 4 had a mean glomerular diameter of 105.3 ± 2.0 $\mu$ which was not different from Group 2 ($P = 0.151$) and Group 1 ($p = 0.093$).

The incidence of segmental sclerosis/hyalinosis in each group and at the various points in time is shown in Table 4. At twelve

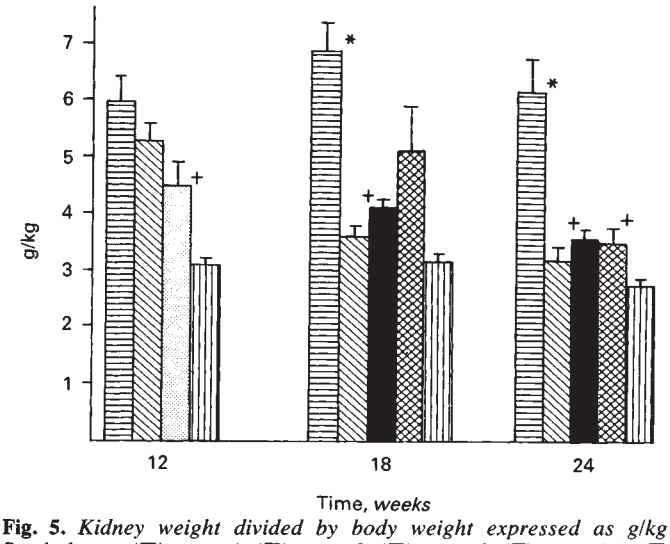


**Fig. 5.** *Kidney weight divided by body weight expressed as g/kg.* Symbols are: (▤) group 1; (▧) group 2; (▨) group 3; (■) group 3A; (▦) group 3B; (▥) group 4. *$P < 0.05$ for group 1 versus group 2. + $P < 0.05$ for group 1 versus 3, 3A or 3B.

weeks all three groups had significant sclerosis compared to controls. But by 18 weeks the incidence of sclerosis had decreased significantly in all groups except group 1. This decrease in the incidence of sclerosis persisted at 24 weeks. The incidence of sclerosis/hyalinosis in the individual animals are shown in Figure 6. Abnormalities of visceral epithelial cells (vacuolization and reabsorption droplets) were the most frequent findings. Relatively few advanced lesions (hyalinosis) were seen at this time (Fig. 7C).

At 18 weeks when the animals were no longer subjected to PA injections, there was a dramatic improvement in histology in groups 2 and 3A/B. The frequency of the lesions were much less and when present, they corresponded to the early stage of evolution of sclerosis (epithelial cell damage). In contrast, the frequency of lesions not only did not change, but they were more advanced (a mixture of epithelial damage and hyalinosis with collapsed capillaries) in group 1.

At 24 weeks, group 1 kidneys showed further progression of the lesions, whereas the improvement in groups 2 and 3A/3B persisted with only a few areas of hyalinosis (Fig. 8).

Huge tubular PAS-positive casts and dilated tubules were seen frequently, and in the most severely affected kidneys (group 1 at 24 weeks). There was focal tubular atrophy with interstitial lymphocytic infiltration occasionally disrupting the tubular basement membrane and extending among the tubular epithelial cells. There were no hypertensive arteriolar changes seen.

The ultrastructural findings in all proteinuria groups were similar. The foot processes of glomerular visceral epithelial cells were completely or almost completely effaced (Fig. 9A). In contrast, there was restitution of the normal appearance in animals in group 2 (Fig. 9B). Other electron microscopic abnormalities corresponded to those in the light microscopic preparations in all groups at all stages of the experiments.

There was significant heterogeneity in histology among the individual animals in each group (Fig. 6). There was good correlation between the extent of tubulointerstitial lesions and the degree of proteinuria, gross appearance and weight of the kidneys, whereas such correlation with the glomerular lesions was poor.

### *Immunofluorescence*

The pattern of immunofluorescence for albumin, fibrin, complement (C3), and IgG corresponded to the reabsorption droplets of epithelial cells. The immunofluorescence was always most intense at 12 weeks, with a progressive decrease thereafter. The immunofluorescence for albumin was always brighter. There was a trend for more intense immunofluorescence in group 1 versus group 2. IgM was localized to the mesangium in a granular pattern with an intensity of trace to 2+. There was a progressive increase from 12 weeks to 24 weeks.

## Discussion

This study used the chronic form of PAN to examine the effects of prolonged CEI and low protein diet on the development of focal segmental glomerular sclerosis. Chronic PAN is induced by repeated aminonucleoside injections and is characterized by heavy persistent proteinuria and FSH in 7 to 12% or more of the glomeruli depending on the particular study and the duration of follow-up of the disease [8, 9, 16]. It is also possible to induce a similar chronic form with only one intravenous injection of PA [23].

The two diets used were isocaloric and had the same content of phosphorus, calcium and magnesium, in order to exclude any possible effect of these factors on any beneficial effect from a low protein diet [24, 25].

The most interesting finding of this study is that whereas low protein diet and CEI do not affect the course of chronic PAN as long as the injections of PA continue, they both result in a marked improvement in both proteinuria and histologic appearance if they are continued after the completion of the PA injection schedule. A protective effect of dietary protein restriction in chronic aminonucleoside nephrosis using the one injection model has recently been reported [26]. But in that study only one time point, 18 weeks, was examined.

By sacrificing animals from the various groups at multiple points in time, the observation was made that CEI and low protein diet not only prevented or arrested but actually reversed the proteinuria and early sclerosis of this model. In the untreated group, the damage to the kidneys persisted and progressed despite the cessation of the aminonucleoside injections.

Comparison of the low protein diet alone, CEI alone and low protein diet plus CEI demonstrated that the low protein diet throughout the experiment initially improved and then completely reversed proteinuria as well as reversing the histologic lesions of the kidneys to a very significant degree. Treatment with CEI alone throughout the experiment did not affect proteinuria during the first 12 weeks, whereas it decreased it significantly during the last 12 weeks, even though this effect was slower and less prominent than with the low protein diet. Adding a low protein diet to CEI for the last 12 weeks caused a more precipitous and complete reversal of proteinuria.

It is true that the degree of sclerosis in the CEI group at 12 weeks was not as high as in the other two experimental groups (7% vs. 16% and 15%) or as that seen with the same treatment in our previous study (13%) [16]. However, it was still significantly higher than in the age-matched controls. It is possible

**Table 4.** Incidence of sclerosis

| | Group 1 | Group 2 | Group 3 | | Group 4 |
|---|---|---|---|---|---|
| | | | Group 3A | Group 3B | |
| 12 weeks | 16 ± 1.2% (N = 6) | 15 ± 1.3% (N = 5) | 7 ± 1.3%[a] (N = 5) | | 1.3 ± 0.4%[a] (N = 3) |
| 18 weeks | 13.6 ± 1.5% (N = 6) | 3.3 ± 1.6%[a,c] (N = 7) | 2 ± 0.4%[a,b] (N = 6) | 3 ± 0.9%[a] (N = 5) | 1.5 ± 0.0%[a] (N = 2) |
| 24 weeks | 18.1 ± 2.6% (N = 7) | 5.4 ± 2.5%[a,c] (N = 8) | 3.3 ± 0.8%[a,b] (N = 6) | 4 ± 0.8%[a,b] (N = 7) | 0.6 ± 0.5%[a] (N = 4) |

[a] $P < 0.05$ from group 1
[b] $P < 0.05$ group 3 at 12 weeks versus group 3A and 3B at 18 and 24 weeks
[c] $P < 0.05$ group 2 at 18 and 24 weeks versus 12 weeks

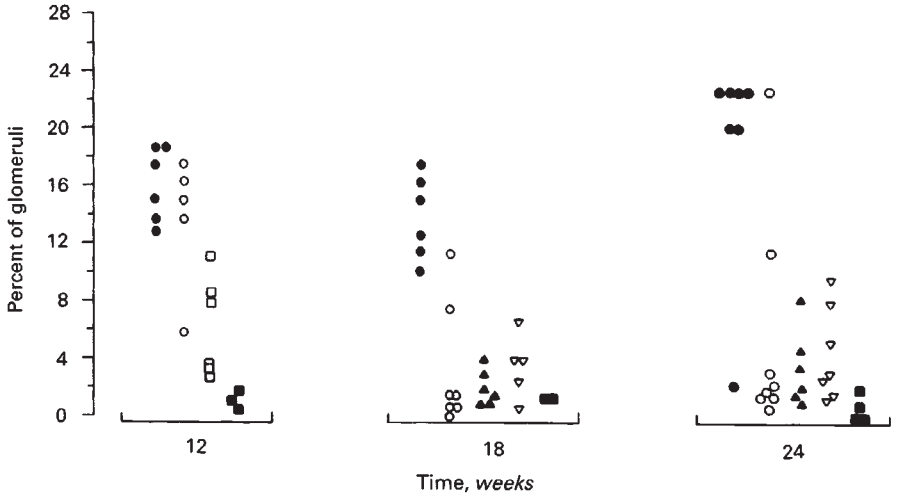


**Fig. 6.** *Incidence of glomerulosclerosis per animal at the three time points of sacrifice.* Results are expressed as the percentage of glomeruli involved with sclerosis in the individual animal. Symbols are: (●) group 1, (○) group 2, (□) group 3, (▲) group 3A, (△) 3B, (■) group 4.

that this difference is due to sampling error since proteinuria in the untreated groups and the enalapril-treated groups in both studies was the same. There was wide scatter in incidence of sclerosis among the individual animals in most groups (Fig. 6).

It is obvious that the early lesions in the sequence of FSH can be reversed by appropriate treatment. The exact point beyond which there is no reversibility is not clear. However, it is possible that the "hyalinosis" is resolvable because such lesions were observed in seven out of nine kidneys in group 2 at 12 weeks, whereas they were not seen in any kidneys at the 18-week sacrifice and in only seven out of 16 kidneys at 24 weeks.

The improvement in glomerular structure was associated with amelioration of the tubulointerstitial damage and return of the kidney weight to body weight ratio to normal, suggesting that renal hypertrophy was reversed. The finding of significantly smaller glomerular diameters at 24 weeks in the low protein diet animals compared to PAN animals also would suggest reversal of glomerular hypertrophy. Whether this overall effect is secondary to the glomerular healing or a concomitant direct effect of either treatment on the tubular lesions is not clear. The latter possibility is raised because of evidence that dietary protein content and angiotensin II have regulatory effects on tubular epithelial growth and regeneration [27, 28].

Differences in systolic blood pressure do not seem important enough to explain the difference in histology among the various groups. No arterial or arteriolar abnormalities consistent with systemic hypertension were observed in the kidneys. The changes in proteinuria and histology are concomitant and it is unlikely that variations in serum protein levels had a significant additional effect on the proteinuria. The immunofluorescent findings were similar in all groups and non-specific, consisting of protein reabsorption droplets which represented the effects of the non-selective proteinuria. IgM was present in the mesangium, most strongly in Group 1 and was probably related to the development of FSH.

The exact mechanisms through which low protein diet or CEI cause improvement in FSH cannot be answered by this study. Single nephron hyperfiltration and/or intraglomerular hypertension could be considered as the causative mechanisms amenable to correction by either of these treatments [29, 30]. However, the intraglomerular hemodynamics in chronic PAN are not known with certainty. One study has shown increased intracapillary hydrostatic pressures nine weeks after induction of chronic PAN (with a single intravenous PA injection, 50 mg/kg) [31], whereas in another study, repeated measurement in the same glomeruli over eight weeks showed normal or even low intraglomerlar capillary pressures [32]. In addition, recent studies doubt the importance of intraglomerular hemodynamics in the pathogenesis of FSH [33]. An alternative hypothesis is that FSH is related to the degree of preceding glomerular hypertrophy regardless of the intracapillary hydrostatic pressures or single nephron glomerular filtration rate [34, 35]. Recently it has been shown that CEI decreases glomerular

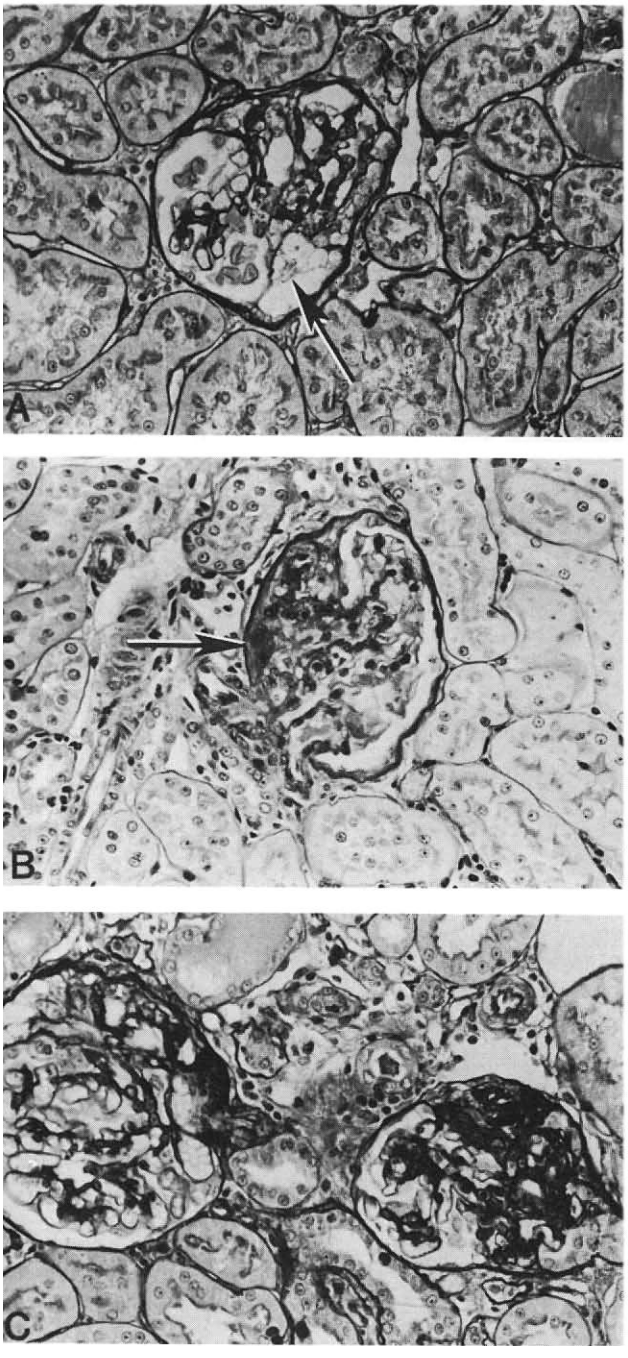


**Fig. 7.** *Light microscopy at 12 weeks showing an early* (**A**)*, intermediate* (**B**) *and advanced* (**C**) *lesion.* **A** shows an early lesion with segmental enlargement and coarse vacuolization of visceral epithelial cells (arrow). Capsular adhesions are also present. **B** shows an intermediate lesion with a small group of capillaries obliterated mainly by plasma protein insudative lesion ("hyalinosis") (arrow). There is also slight increase in mesangial matrix. **C** shows an advanced lesion with extensive capillary luminal loss by combined insudative lesions and increased mesangial matrix-basement membrane material affecting only one of two glomeruli. (PAS × 200)

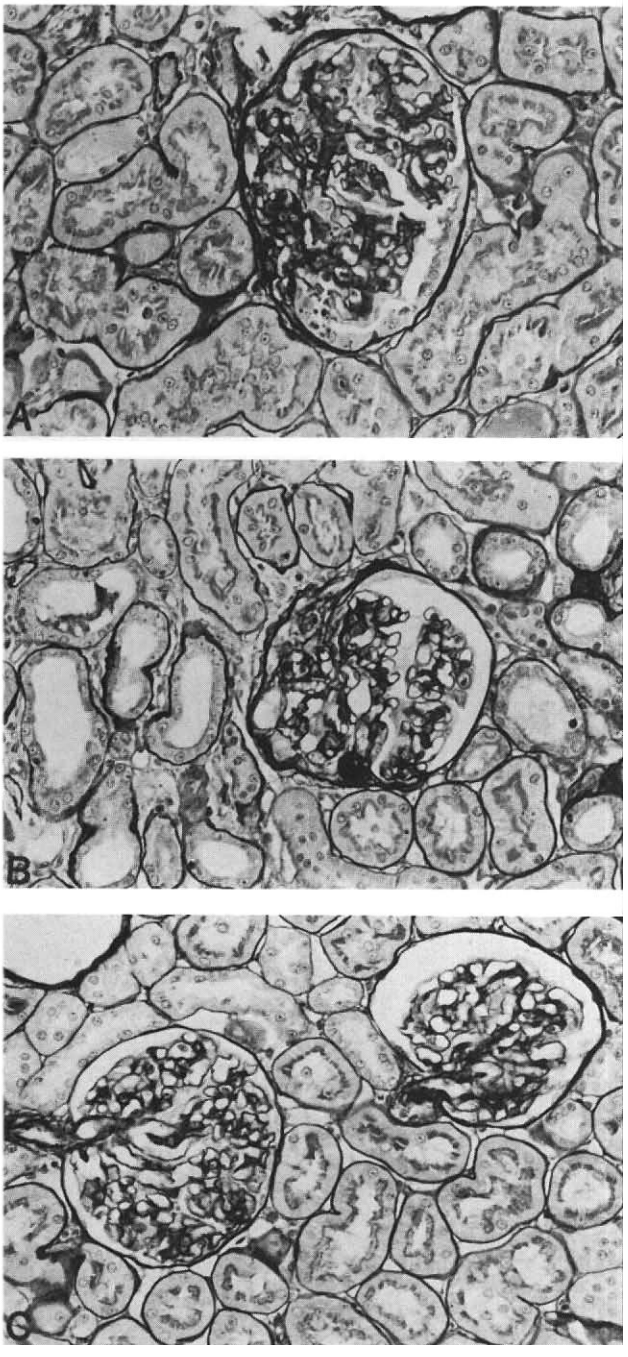


**Fig. 8.** *Light microscopy at 24 weeks in group 1* (**A**)*, group 2* (**B**) *and group 4* (**C**) *animals.* **A** is a group 1 animal showing a glomerulus with intermediate to advanced segmental sclerosis. **B** is group 2 animal with a small segment of sclerosis with "hyalinosis" in this glomerulus. **C** is a group 4 (control) animal with glomeruli that are without segmental sclerosis. (PAS × 200)

surface area (a measure of glomerular hypertrophy) in normal as well as in partially nephrectomized rats, and that it decreases mesangial cell proliferation in vitro [36].

Experiments on growth of tubular epithelial cells in vitro showed that angiotensin II enhances the mitogenic effects of epidermal growth factor on these cells (both angiotensin II and EGF used at "physiologic" concentrations) [28]. Finally, it has been shown that dietary protein intake in the rat is directly related to plasma renin activity, angiotensin I and aldosterone

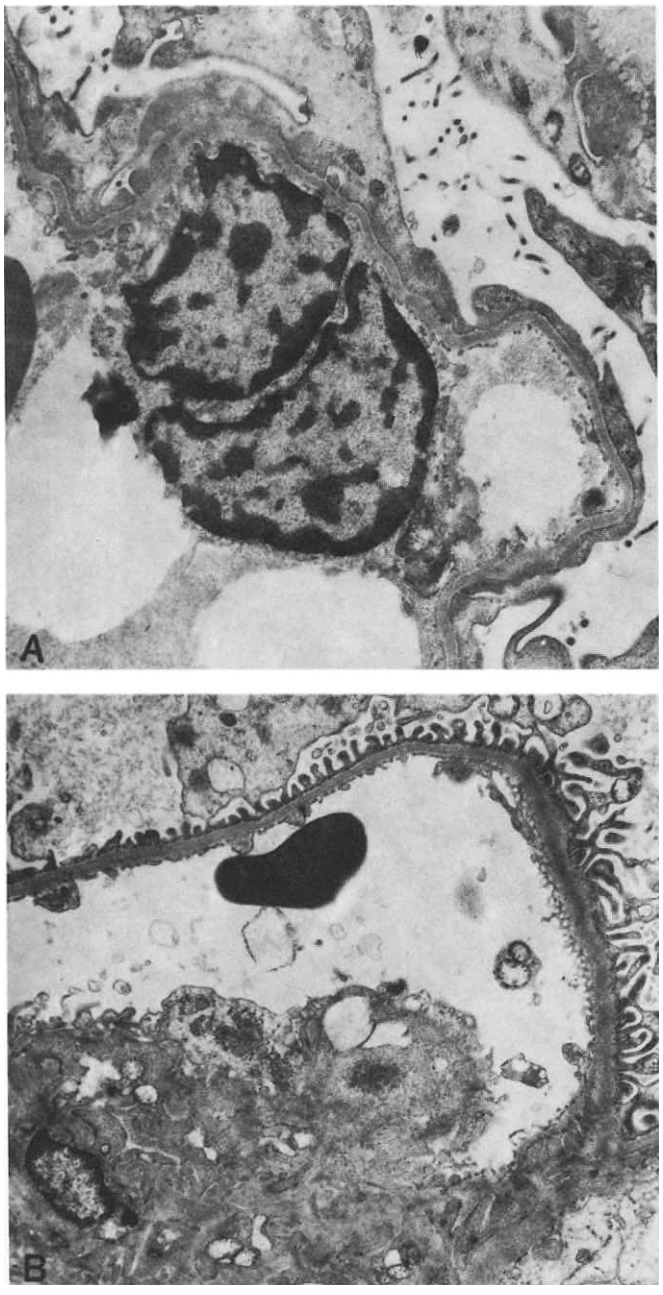


**Fig. 9.** *Electron microscopy of a group 2 animal at 12 weeks* (**A**) *and 24 weeks* (**B**). **A** is an electron micrograph of group 2 animal at twelve weeks. There is complete effacement of the foot processes of epithelial cells; note cytoplasmic microvilli in the urinary space. (× 3200). **B** is an electron micrograph of group 2 animal at 24 weeks. The foot processes are now well preserved. (× 2500)

levels as well as vasodilatory prostaglandin levels and is associated with renal hypertrophy [27].

Considering all these data, it is conceivable that low protein diet and CEI attenuate hypertrophic and regenerative processes in the glomeruli as well as in the tubules. A similar effect on both glomerular and tubular epithelial cells may be more likely given the common embryologic origin of these two kinds of cells.

It is possible then that in chronic PAN, low protein diet acts by decreasing the activity of the renin-angiotensin system in the kidney, thereby decreasing the hypertrophic/regenerative response of the injured podocytes and possibly of the mesangial cells as well. To the extent that there is indeed a link between degree of hypertrophy and sclerosis, the profound amelioration of sclerosis seen in this experiment can also be explained.

The fact that the response to the low protein was more prominent than to enalapril alone may indicate additional effects of low protein on above processes, possibly through changes of renal prostaglandin levels [27, 28], or decrease of oxidant stress per nephron [37], or through decreased levels of various growth factors.

The outcome of this study raises the possibility that use of low protein diet and/or CEI in clinical renal disease with FSH may allow the reversal of the self-perpetuating process towards end-stage renal failure and healing of the early FSH lesions.

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