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Sex related differences in glomerular ultrafiltration and proteinuria in Munich-Wistar rats

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Sex related differences in glomerular ultrafiltration and proteinuria in Munich-Wistar rats. Munich-Wistar rats (MWF/Ztm), originally selected for high number of superficial glomeruli, were used to correlate abnormal urinary protein excretion with glomerular hemodynamics and glomerular morphology. Two animal groups were used, one of male and one of female rats. They were kept periodically in metabolic cages to determine urinary protein excretion. All animals were fed standard rat chow. In male animals protein excretion, evaluated at seven weeks of age, was already significantly higher than in females (17 ± 11 vs. 8 ± 3 mg/24 hr), and then progressively increased averaging 291 ± 51 mg/24 hr at week 21. In females urinary protein excretion was within the normal range up to week 18 and averaged 25 ± 13 mg/24 hr at week 21. Body and kidney weight at the end of the experimental period were significantly higher in males than in females. Whole kidney inulin clearance (C_{In}) and single nephron glomerular filtration rate (SNGFR) were significantly higher in male than in female rats, while mean glomerular capillary hydraulic pressure ($\bar{P}_{GC}$) and transcapillary hydraulic pressure difference ($\overline{\Delta P}$) were comparable. Single nephron glomerular plasma flow (Q_A) and afferent and efferent arteriolar resistance were comparable in male and female rats. The calculated glomerular ultrafiltration coefficient (K_f) was significantly higher in male than in female MWF/Ztm rats. No significant differences were detected between the two groups in the total number of glomeruli, and in glomerular size. These findings indicate that male MWF/Ztm rats develop spontaneous proteinuria, which progressively increases with the age. This abnormal urinary protein excretion, not observed in female rats, is associated with higher SNGFR and elevation of K_f, without significant changes in the other determinants of glomerular ultrafiltration. These observations, together with the results of the morphometrical analysis, indicate that hydraulic membrane permeability is elevated in male rats when compared to females. It is speculated that the defects in membrane permeability to water and macromolecules are genetically determined rather than a consequence of glomerular hemodynamic changes.

An abnormal passage of plasma proteins across the glomerular capillary wall to the urinary space is the common event in many nephropathies in which glomerular membrane permselective properties are altered by the underlying disease. Several animal models have been used to study the mechanisms responsible for the loss of glomerular selective properties [1–5]. Common findings in these models are massive proteinuria and subsequent progression of the disease to glomerulosclerosis and renal insufficiency. However, these models are only poorly representative of human disease states due to the artificial way in which the desired glomerular dysfunction is obtained. A new model of sex-related spontaneous proteinuria has recently become available. A rat strain, originally selected from Munich-Wistar rats by Frömter for a high number of superficial glomeruli (MWF/Ztm), has been found to have elevated urinary protein excretion rate as compared to other strains of rats, males having significantly higher urinary protein excretion values than females [6]. These animals represent a unique model of spontaneous proteinuria which can provide important information on the mechanisms of proteinuria as well as on the long-term effects of the increased plasma protein traffic across the glomerular capillary wall. It has been reported by Hackbarth et al [7] that male MWF/Ztm rats have a reduced glomerular density, together with increased glomerular size, with respect to another strain of rats. Basing on these observations, Halbach et al [8] suggested that the reduced glomerular density in these rats was the cause of the observed glomerular hyperfiltration, which in turn may induce changes in permselectivity function and damage of the glomerular structure. The increased transglomerular protein flux was believed to further contribute to the damage of the glomerular membrane. These same authors also suspected that proteinuria in MWF/Ztm rats could be related to the presence of superficial glomeruli.

The aims of the present study were: 1) to establish whether in males, as compared to females MWF/Ztm rats, the increased urinary protein excretion was associated with hemodynamic changes; and 2) to investigate the relationship between protein excretion rates and glomerular morphometrical parameters, such as glomerular density and size, and the number of superficial glomeruli.

Methods

Munich-Wistar rats MWF/Ztm, originally supplied by H. Hackbarth from Hannover (BRD), were bred and raised in our facilities. Two groups of 16 animals eacn were studied. The first group consisted of male rats while female rats comprised the second group. All animals were fed normal (20%) protein diet [2], and they received food and water ad libitum. After the seventh week of age, urinary protein excretion was measured weekly by collecting urine over a 24 hour period using metabolic cages. Total protein concentrations in urine were determined by the modified Comassie blue G dye-binding method [9]. Two additional groups of six male Wistar rats (body weight ranging respectively 180 to 210 g and 280 to 350 g, supplied by

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Charles River Laboratories, Calco, Italy) were used as reference groups for urinary protein excretion measurements.

Micropuncture studies

Eight rats from each group, aged sixteen to twenty-one weeks old for the male group and eighteen to twenty-one weeks old for the female group, underwent micropuncture. They were anesthetized with Inactin (100 mg/kg body weight, intraperitoneally), placed on a constant temperature micropuncture table at 37 to 38°C, and tracheostomized. Immediately after the induction of anesthesia, the left femoral artery was catheterized with PE-50 polyethylene tubing, and blood was sampled for baseline determination of hematocrit and plasma protein concentration. This catheter was used for subsequent periodic blood sampling and for mean arterial pressure ($\overline{AP}$) measurement, by means of an electronic pressure transducer (Model P23ID, Statham Instrument Division, Gould Inc., Oxnard, California, USA) connected to a writing recorder (Battaglia Rangoni, Bologna, Italy). Polyethylene catheters were also inserted into the left jugular and left femoral vein for infusion of inulin and para-aminohippurate (PAH) solution and isoncotic rat plasma. Intravenous infusion of inulin (7%) and PAH (0.2%) dissolved in 0.9% NaCl was started at a rate of 1.2 and 0.8 ml/hr, respectively, for male and female rats. The left kidney was then exposed through a subcostal incision and separated from adrenal gland and surrounding perirenal fat. A PE-10 catheter was inserted into the left ureter and the kidney was placed in a Lucite holder, bathed with isotonic NaCl and its surface illuminated with a fiber-optic light source. Isoncotic rat plasma, obtained from a littermate immediately before the operation, was infused during the experiment to compensate surgically induced loss of plasma volume [10], using the following protocol. During surgery a total amount of 1% of body weight of isoncotic rat plasma was infused over a period of 45 minutes, followed by constant infusion at 0.4 ml/hr for the duration of the experiment.

After one-hour equilibration period, micropuncture measurements and clearances were started. Tubular fluid was collected from surface proximal convolutions of five to six separate nephrons with sharpened glass pipettes, with external diameter of 10 to 12 μm, during an exactly timed collection period of 1.5 to 3 minutes. Three to five samples of efferent arteriolar blood were withdrawn from superficial star vessels using sharpened oil-filled heparinized pipettes (14 to 16 μm OD). Blood samples were obtained at the midpoint of each clearance period, coincident with the microsamples collections, for determination of hematocrit and plasma concentrations of inulin, PAH and total proteins.

Time-averaged hydraulic pressure was measured in randomly-chosen surface glomerular capillaries, proximal tubules, and efferent arterioles using sharpened glass pipettes (2 to 4 μm OD) and a servo-nulling micropipet pressure system (Model 5, Instrumentation for Physiology and Medicine, San Diego, California, USA). Hydraulic output from the servo-nulling system was connected to a second pressure transducer and to the writing recorder [11].

Colloid osmotic pressures of plasma in afferent (Π_A) and efferent (Π_E) arterioles were estimated from protein concentration values in femoral arterial (C_A) and efferent arteriolar (C_E) plasma samples, respectively, using the equation derived by Deen et al [12]. These estimates of preglomerular and postglomerular plasma oncotic pressure were used to calculate single nephron filtration fraction (SNFF). SNGFR, Q_A, afferent and efferent arteriolar resistances (R_A and R_E) were calculated using the equations reported in literature [12]. Calculation of K_f was performed according to the model of glomerular ultrafiltration of Deen et al [13]. Mean values of inulin and PAH clearance were calculated using usual formulas and expressed as ml/min/g of kidney weight, in order to take into account the observed difference in kidney size between male and female rats.

Analytical

The volume of tubular fluid samples was measured from the length of the fluid column in a constant bore capillary tube of known internal diameter. Inulin concentration in tubular fluid was determined by the microfluorescence method of Vurek and Pegram [14] using a fluoromicrophotometer (American Instrument Co., Silver Spring, Maryland, USA). Determinations were usually performed in duplicate. Inulin and PAH concentrations in plasma and urine samples were measured respectively using the macro-anthrone method of Fuhr, Kaczmarczyk and Kruttgen [15] and the method of Smith, Finkelstein and Aliminosa [16].

Protein concentrations in efferent arteriolar and femoral arterial plasma samples were determined, usually in duplicate, using an ortho-phthalaldehyde technique [17] adapted for small sample volumes as described by Viets et al [18].

Morphometry

At the end of the micropuncture experiment the left kidney was removed and weighed. The kidney was cut transversely at the ilus, and one of the two parts was processed for light microscopy as previously described in detail [19]. Embedding of the specimens was done to obtain tissue sections corresponding to the maximal transverse section of the kidney. Morphometric analysis was performed in each animal that underwent micropuncture, using the technique described by Hackbarth et al [7], with slight modifications. Briefly, histological sections were projected by a Zeiss microscope, with an enlarging factor of 35 to 55, on a plastic sheet. Exact enlargement was calculated from the direct measure of the renal cortex diameter obtained with a micrometer eyepiece (Nachet, Cedex, France) and the measure of the corresponding diameter on the projected image. The renal cortex was then outlined and its cross sectional area determined by weighing the plastic sheet inside the outlined zone. The total number of glomeruli in the section was counted, and the density of glomeruli was calculated as number of glomeruli per mm^2. Because, as stated by stereological principles [20], area density equals volume density, the measured glomerular density is a direct estimation of the number of glomeruli per unit volume in this zone of the renal cortex. On the same sections the total number of glomeruli in direct contact with the renal capsule was counted.

Glomerular size was examined in at least 20, randomly chosen glomeruli per animal. Using the micrometer eyepiece the diameter of the Bowman's capsule was measured in two random perpendicular directions, and the average diameter (D_{BC}) was then calculated. These measurements were repeated in the same glomeruli for the capillary tuft (D_{CT}). Mean volumes of Bowman's capsules (V_{BC}) and capillary tufts (V_{CT}) were

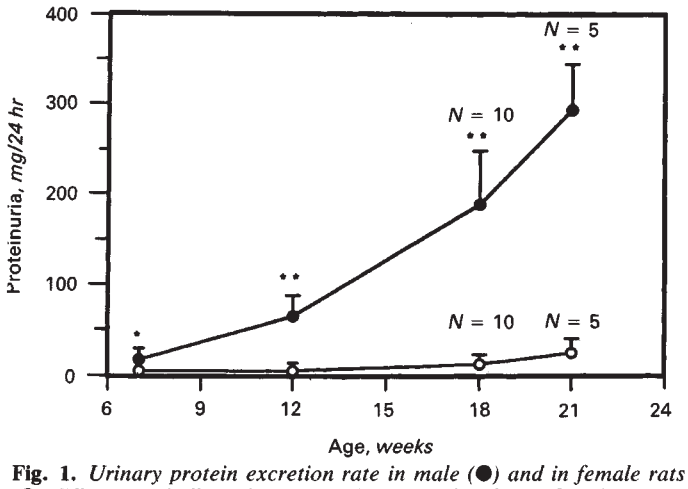

Fig. 1. *Urinary protein excretion rate in male (●) and in female rats (○).* Where not indicated, $N = 16$. * $P < 0.05$ male vs. female group. ** $P < 0.001$ male vs. female group.

calculated from the respective mean diameters using the equation derived by Weibel [21]:

$$V_i = \frac{\beta}{k}\left(\frac{D_i^2\,\pi}{4}\right)^{3/2}$$

were k = 1.1 is a distribution coefficient, $\beta = 1.38$ is a coefficient that takes into account the spherical shape [21, 22], and D_i stands for Bowman's capsule diameter or capillary tuft diameter.

Statistics

All values are expressed as mean ± standard deviation. Statistical significance between individual group means was calculated using unpaired Student's *t*-test or Rank-sums test where appropriate. Statistical significance was defined as $P < 0.05$.

Results

Urinary protein excretion

Urinary protein excretion, measured periodically in the two animal groups, is reported in Figure 1. At seven weeks of age, protein excretion in male rats was already significantly elevated, when compared to values observed in female rats. Consequently male rats developed massive proteinuria, reaching the value of 291 ± 51 mg/24 hr at week 21. Protein excretion in female rats remained always significantly lower than in males, and showed only a tendency to increase at 21 weeks of age (25 ± 13 mg/24 hr). Urinary protein excretion in the two groups of normal Wistar rats was comparable to the values measured in female MWF/Ztm rats (averaging 10 ± 3 and 28 ± 10 mg/24 hr for the youngest and the oldest rats, respectively). These results are consistent with the protein excretion measurements reported by Alt, Maess and Hackbarth in the same strain of rats [6]. This massive proteinuria in male rats is likely of glomerular origin, as previously documented by Halbach et al [8]. Mean body weight (Table 1) at the end of the experimental period (18 ± 3 weeks for males, 20 ± 1.9 weeks for females) was found to be significantly higher in male rats. This difference in growth took place despite total animal food and water intake, measured periodically when the animals were kept in metabolic cages, which was not significantly different between the two groups (data not shown).

Micropuncture studies

Table 1 summarizes the mean values of renal and single nephron functional parameters measured during the micropuncture experiment. Mean kidney weight was significantly higher in male than in female rats, reflecting the same difference in body weight observed in the two groups. Hematocrit was slightly but significantly lower in female rats. Mean arterial blood pressure ($\overline{AP}$) during the micropuncture experiment was about 5 mm Hg higher in the male group. For both groups these values were higher than those commonly found in normal Munich-Wistar rats [5, 10]. These data were consistent with systolic blood pressure measurements obtained in two additional groups of male and female MWF/Ztm rats, which averaged 157 ± 6 mm Hg ($N = 6$) and 147 ± 13 mm Hg ($N = 6$), respectively. In contrast, values obtained in normal Wistar rats averaged 133 ± 9 mm Hg ($N = 6$). Hydraulic pressures in glomerular capillaries ($\bar{P}_{GC}$), in proximal tubules (P_T) and in efferent arterioles (P_E) were not different in the two groups. As a result, also mean glomerular transcapillary hydraulic pressure ($\overline{\Delta P}$) was found comparable in male and female rats. Plasma protein concentrations in afferent as well as in efferent arterioles (C_A and C_E) were estimated to be comparable in the two groups. SNGFR in male rats was found approximately 60% higher than in females. Mean Q_A was numerically increased in the same animal group, although the difference did not reach statistical significance, while SNFF was elevated in male rats. Mean values of R_A and R_E were slightly higher in female group, without reaching statistical significance. Calculation of unique values of ultrafiltration coefficient (K_f) was possible because filtration pressure disequilibrium was observed in all animals. The mean value of K_f calculated for the male group was approximately 70% higher than in female rats.

Regarding whole kidney functional parameters, mean C_{In} was significantly higher in the male group. This difference persisted even when C_{In} was corrected by kidney weight. Total kidney PAH clearance (C_{PAH}) in male rats was significantly higher than in females; when C_{PAH} was normalized for kidney weight, this difference did not reach statistical significance. Mean FF was reduced in female group, but still the difference was not significant.

Morphometry

The results of the morphometrical analysis are summarized in Table 2. As expected, the cross sectional area of the renal cortex, measured in the transverse section of the kidney, was found to be proportional to the kidney weight, and was thus reduced in female rats by approximately 40%. Despite the difference in kidney size with sex, the total number of glomeruli counted in the transverse section was only slightly decreased in female rats, but the difference was not statistically significant. As a consequence, glomerular density was significantly higher in female than in male rats. The findings that male and female rats have a comparable number of glomeruli and that the ratio of whole kidney C_{In} between male and female rats was higher than

Table 1. Whole kidney and single nephron functional parameters

Group	Body wt	K wt	Ht	$\overline{AP}$	$\overline{P}_{GC}$	P_T	$\overline{\Delta P}$	P_E	C_A	C_E	Π_A	Π_E
	g		%	*mm Hg*					*g/dl*		*mm Hg*	
Male (N = 8)												
Mean ± SD	317	1.33	49.5	131.8	49.6	13.5	35.5	19.9	5.1	7.6	16.2	29.1
	53	0.13	1.2	4.6	1.4	0.9	2.1	1.2	0.5	0.5	1.6	3.7
Female (*N* = 8)												
Mean ± SD	218	0.86	47.7	126.3	50.2	13.2	37.0	20.5	5.2	7.4	16.6	28.3
	11	0.11	1.4	5.2	2.0	0.9	2.0	1.2	0.3	0.5	1.5	3.3
P value[a]	<0.001	<0.001	<0.05	<0.05	NS	NS	NS	NS	NS	NS	NS	NS

Abbreviations are: K wt, kidney weight; $\overline{AP}$, mean arterial pressure: $\overline{P}_{GC}$, mean glomerular capillary hydraulic pressure; P_T, proximal tubule afferent and efferent arteriolar plasma protein concentrations; Π_A and Π_E, afferent and efferent arteriolar oncotic pressures; Q_A, single nephron
[a] Males vs. females

the corresponding ratio of SNGFR would indicate that the difference in SNGFR between the two groups is higher in deep than in superficial glomeruli.

The number of superficial glomeruli counted in each transverse section was almost double in female animals, confirming the observation recorded during the micropuncture experiments that female rats seem to have a higher number of superficial glomeruli. Direct estimation of glomerular size revealed comparable results in the two animal groups; in fact, no differences were found in the mean volume of Bowman's capsules or in the mean volume of capillary tufts.

Discussion

We have confirmed that in MWF/Ztm male rats urinary protein excretion rate is abnormally elevated. Protein excretion values recorded at seven weeks of age were already significantly elevated in males than in females, and progressively increased with age, reaching a mean value of 291 ± 51 mg/24 hr at week 21. In contrast, female rats had values for urinary protein excretion within the normal range with a tendency to increase at the end of the observation period (that is, week 21), which only reached mean value of 25 ± 13 mg/24 hr. The abnormal glomerular permeability to proteins observed in male MWF/Ztm rats was associated with higher mean body and kidney weight in comparison to females, despite a comparable intake of food and water.

In a rat model of extensive renal ablation [1] micropuncture studies showed a higher SNGFR than in control rats, accompanied by an increase in glomerular plasma flow and transcapillary hydraulic pressure difference. It has been postulated that these changes lead to progressive loss of selective barrier properties resulting in proteinuria [23–25]. These findings prompted us to evaluate whether changes in glomerular hemodynamics in our male MWF/Ztm rats, as compared to females, would explain the abnormal glomerular permeability to proteins. Our micropuncture study demonstrated that mean SNGFR in male rats is approximately 60% higher than in females, and that Q_A tends to increase in the male group, although the difference did not reach statistical significance. At variance with the above mentioned models, in our experimental conditions hyperfiltration in male rats was not associated with glomerular capillary hypertension. Actually, despite the fact that the mean blood pressure was slightly higher in male than in female rats, transmembrane hydraulic pressure differences were comparable in both groups of animals. The calculated values of K_f, the product of glomerular capillary filtering surface area and hydraulic membrane permeability, were significantly higher in male than in female animals. Since male rats showed a significant increase in kidney weight, we wondered whether the elevation in K_f values in male rats was simply due to an increase in filtering surface area or to alterations in effective membrane permeability. However, no differences were detected in glomerular size between male and female rats as measured by mean volume of Bowman's capsules and capillary tufts. It is reported in literature [22, 26] that glomerular capillary surface area, as measured by standard stereological techniques, is generally proportional to glomerular volume. Based on this observation, we can assume that glomerular capillary surface area is also comparable in male and female rats in our experimental conditions. Eventually, if the increased plasma protein traffic across the membrane observed in male rats, when compared to females, would increase glomerular membrane thickness and/or mesangial volume, we would expect these changes to reduce filtering surface area in these animals. Thus, the significant elevation in K_f in male rats is likely the consequence of an increase in effective hydraulic permeability of the capillary wall. A study is in progress in our laboratory to quantify sex related differences in glomerular structures in these experimental conditions.

Our present results indicate a possible genetic and sex related defect in glomerular membrane constituents which leads to higher hydraulic permeability and to loss of permselective functional properties. An alternative possibility to explain our findings is that an increase in SNGFR itself, in male over the female rats, could induce changes in glomerular membrane properties. As proposed by other authors [27], glomerular hyperfiltration could increase glomerular transcapillary convective flux of plasma proteins, leading to progressive damage of the glomerular membrane. However, previous experiments have indicated that intracapillary hypertension, rather than hyperfiltration per se, has deleterious effects on glomerular structure [3, 25, 28]. Actually the pharmacological control of intracapillary hypertension can repair glomerular structural lesions even when hyperfiltration is maintained [28]. For these reasons we consider that changes in glomerular permeability in our male rats are the consequence of the selective increase in SNGFR unlikely, and rather favor the possibility of a congenital defect leading to a progressive loss of glomerular membrane permeability. The difference in urinary protein excretion values

Table 1. *Continued*

SNGFR	Q_A		R_A	R_E	K_f	C_{In}	C_{PAH}	C_{In}	C_{PAH}	
nl/min		SNFF	$10^{10} \cdot$ *dyne sec cm*$^{-5}$		*nl/sec/mm Hg*	*ml/min*		*ml/min/g K*		FF
50.7	151	0.33	2.5	1.2	0.067	1.21	3.39	0.91	2.52	0.35
8.6	41	0.02	0.6	0.4	0.023	0.36	0.96	0.22	0.51	0.05
31.3	121	0.29	3.1	1.5	0.038	0.57	1.78	0.66	2.05	0.32
6.5	18	0.04	0.6	0.3	0.011	0.15	0.47	0.13	0.39	0.06
<0.001	NS	<0.05	NS	NS	<0.05	<0.01	<0.05	<0.05	NS	NS

hydraulic pressure; $\overline{\Delta P}$, mean glomerular transcapillary hydraulic pressure difference; P_E, efferent arteriolar hydraulic pressure; C_A and C_E, plasma flow; R_A and R_E, afferent and efferent arteriolar resistance; K_f, ultrafiltration coefficient.

Table 2. Morphometrical parameters of renal cortex, glomerular density and size

Group	Renal cortex cross sectional area *mm*^{2}	Glomerular density *glomeruli/mm*^{2}	Total number of glomeruli per section	Number of superficial glomeruli per section	Bowman's capsule volume	Capillary tuft volume
					$\mu m^3 \cdot 10^{-6}$	
Male (N = 8)						
Mean ± SD	28.64	4.78	134	3.0	2.97	2.66
	4.41	0.99	14	1.6	0.22	0.18
Female (N = 8)						
Mean ± SD	17.47	7.16	124	7.1	2.88	2.47
	2.46	1.15	26	1.4	0.19	0.17
P value[a]	<0.001	<0.01	NS	<0.05	NS	NS

[a] Males vs. females

in males and females, already statistically significant at seven weeks of age, would reinforce this interpretation.

At variance with our present interpretation, Halbach et al [8] suggested that a decreased number of nephrons in male MWF/Ztm rats as compared to other strains could lead to glomerular hyperfiltration and consequently to abnormal protein excretion. However, we counted a comparable number of glomeruli in the transverse section of the kidneys in male and female rats. Assuming that the spatial distribution of glomeruli into the renal cortex is similar to that in the transverse section of the kidney (that is, that the number of glomeruli in this section is proportional to the total number of glomeruli), we can infer that the two animal groups have comparable number of glomeruli per kidney. These observations suggest that the increased urinary protein excretion in male rats is not associated with a reduction in the total number of nephrons.

As far as the possible relationship between proteinuria and superficial glomeruli [8], we counted the number of superficial glomeruli visible in the transverse section of the kidney. The data showed that male rats have lower number of superficial glomeruli than females. Assuming once again that this estimate is proportional to the total number of superficial glomeruli, we can exclude that protein excretion is related to the number of glomeruli in contact with the renal capsule.

We conclude that: 1) in male MWF/Ztm rats a defective glomerular permeability to proteins exists which results in abnormally high proteinuria early in the lifetime; 2) with age, proteinuria progressively increases in males but not in female rats, and is accompanied by higher body weight and kidney size; 3) SNGFR and whole kidney C_{In} are higher in male than in female rats without significant changes in glomerular hydraulic pressures, glomerular plasma flow and plasma protein concentration in afferent and efferent arterioles; and 4) ultrafiltration coefficient (K_f) is higher in males than in females, and is responsible for the selective increase in SNGFR in male rats.

This peculiar strain of rats offers a unique opportunity to further clarify, under the appropriate controlled conditions, the mechanism(s) leading to progressive loss of glomerular permselectivity properties as well as long-term consequences of proteinuria on renal function.

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