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# Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation

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HOSTETTER, T. H., J. L. OLSON, H. G. RENNKE, M. A. VENKATACHALAM, AND B. M. BRENNER. *Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation.* Am. J. Physiol. 241 (Renal Fluid Electrolyte Physiol. 10): F85–F93, 1981.—Micropuncture studies were performed in three groups of male Munich-Wistar rats 1 wk after surgery: *group I*, eight control rats that underwent laparotomy and were fed a normal diet; *group II*, nine rats that underwent right nephrectomy and segmental infarction of five-sixths of the left kidney and were fed a normal diet; and *group III*, seven rats that underwent the same renal ablative procedure and were fed a low protein diet. Single nephron glomerular filtration rate (SNGFR) was higher in the remnant kidney of *group II* rats compared with *group I* rats due to higher average values for mean glomerular transcapillary hydraulic pressure difference ( $\Delta P$ ) and initial glomerular plasma flow rate ( $Q_A$ ) in *group II*. Glomeruli in remnant kidneys of *group II* showed striking alterations in morphology, including epithelial cell protein reabsorption droplets, foot process fusion, and mesangial expansion. *Group III* rats demonstrated a mean SNGFR not statistically different from that of *group I*, but significantly less than that of *group II* rats. This lack of absolute hyperfiltration in remnant glomeruli of *group III* rats relative to *group I* obtained because  $Q_A$  and  $\Delta P$  did not increase above values found in *group I*. The glomerular structural lesions seen in *group II* were also largely attenuated in *group III*. These studies demonstrate that alterations in glomerular hemodynamics associated with renal ablation are accompanied by structural lesions and suggest that sustained single nephron hyperfiltration may have maladaptive consequences by damaging remnant glomeruli.

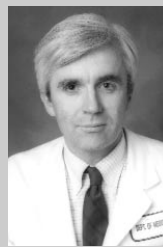
glomerular filtration; hypertrophy; chronic renal failure

WHEN RENAL MASS IS REDUCED, the remaining nephrons undergo functional as well as structural hypertrophy (13). Adaptations in the microcirculation of remnant glomeruli result in an increased mean driving force for filtration and, therefore, a marked increase in filtration rate (8). The magnitude of increase in single nephron glomerular filtration rate (SNGFR) correlates closely with the amount of renal mass that has been lost. That is, greater degrees of ablation result in greater increases of SNGFR in the residual nephrons (17). This functional hypertrophy is generally considered beneficial in the sense that it minimizes the reduction in total glomerular filtration rate that would otherwise occur. However, a number of workers have shown that a pathological process of sclerosis eventually occurs in the glomeruli of these residual nephrons (20, 24, 26, 27). These studies showed that after unilateral nephrectomy and partial ablation of the remaining kidney, eventual sclerotic destruction of remnant glomeruli was accompanied by progressive azotemia, proteinuria, and arterial

## AUTHOR COMMENTARY

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These studies were first submitted as an abstract to the annual combined meeting of the AFCR, ASCI, and AAP. The paper was presented in May 1979 in Washington, DC, at the Sheraton Park Hotel. The old hotel has since been leveled and replaced, but that Monday morning session was packed for the 14 papers that dealt with varied aspects of renal pathophysiology. The presenter remembers the question periods as exhilarating and tough but with occasional similarities to feeding time at the shark tank. The final publication appeared 2 years later. At the initial abstract submission, we provided the descriptions of glomerular hemodynamics and associated morphology only in the subtotal nephrectomized rats and the sham-operated controls. However, the oral presentation reported the third group of rats fed low-protein diets.

The paper represented the combined efforts of the Laboratory of Kidney and Electrolyte Physiology (LKEP) in the department of medicine and laboratories in the department of pathology, all at the Brigham and Women's Hospital. Barry Brenner was the director of the LKEP. During the preceding decade, he and his colleagues had developed the micropuncture techniques and explanatory theory for examining glomerular hemodynamics directly in

hypertension (20, 24, 26). With severe reduction in renal mass, alterations in glomerular structure were detected as early as 2 wk. By 7 wk more than 50% of glomeruli exhibited morphological changes, and all animals died by 90 days (24). In an effort to ascertain the mechanisms that underlie this destructive process, we have attempted to correlate the changes in structure and function that occur in remnant glomeruli at an early stage in this process.

## METHODS

*Glossary*

AP	mean femoral arterial pressure, mmHg
C	protein concentration, g/100 ml
GFR	glomerular filtration rate (whole kidney), ml/min
Hct	blood hematocrit in femoral artery or afferent arteriole, vol/100 ml
$K_f$	glomerular capillary ultrafiltration coefficient, nl/(s · mmHg)
$\Delta P$	glomerular transcapillary hydraulic pressure difference, $P_{GC} - P_T$ , mmHg
$\Pi$	colloid osmotic pressure, mmHg
$\Delta\Pi$	glomerular transcapillary colloid osmotic pressure difference, $\Pi_{GC} - \Pi_T$ , mmHg
P	hydraulic pressure, mmHg
Q	plasma volume flow rate, nl/min
R	resistance to blood flow, dyn·s·cm <sup>-5</sup>
$R_{TA}$	total arteriolar resistance, $R_A + R_E$ , dyn·s·cm <sup>-5</sup>
SNFF	single nephron filtration fraction
SNGFR	single nephron glomerular filtration rate, nl/min

*Superscript*

— (overbar) mean value

*Subscripts*

A	afferent arteriole
E	effluent arteriole
GC	glomerular capillary
T	proximal tubule

Male Munich-Wistar rats weighing 200–250 g were employed in these studies. Three groups of rats were studied. *Group I*: control rats ( $n = 8$ ) underwent laparotomy and manipulation of the renal pedicles and were maintained on standard rat chow consisting of 24% protein (Wayne Lab-Blox, Allied Mills, Chicago). *Group II*: these rats ( $n = 9$ ) underwent right nephrectomy and infarction of approximately five-sixths of the left kidney by ligation of two or three branches of the renal artery. They were also maintained on the same standard rat chow. *Group III*: these rats ( $n = 7$ ) underwent the same renal ablative procedure as *group II* but were fed a 6% protein diet replete with electrolytes, trace minerals, and vitamins for 1–2 wk prior to surgery and were continued on this diet to the time of micropuncture study. All animals were studied 7 days after renal ablation or sham surgery.

On the morning of micropuncture study, the rats were anesthetized with Inactin (100 mg/kg body wt i.p.). They were

rats (1,2). With the use of these approaches, glomerular pressures and flows had been analyzed in a number of physiologic and pathophysiologic states. In addition, the LKEP had developed a strong interest in the permselective properties of the glomerular capillary with respect to macromolecules. With the use of modified clearance techniques and dextran probes, sieving curves were constructed. In this way, the size- and charge-selective defects in the glomerular capillaries had been explored in several disease models. Furthermore, the interaction between hemodynamic forces and the transit of macromolecules had been defined through the mathematical modeling of Bill Deen and Mike Bohrer and then tested in the laboratory. Tom Hostetter entered this exciting environment in the summer of 1977 as a research fellow, 1 yr after Brenner had moved from San Francisco to Boston. His first assignment was to investigate experimental diabetes with an eye toward understanding its proteinuric consequences through sieving studies. On the basis of the work of Andres, Ditzel, Mogensen, and Parving, it then was known that renal hemodynamic alterations occurred in early type 1 diabetes mellitus (3). So, in preparation for examining the permselective properties in the diabetic glomerulus, Hostetter learned glomerular micropuncture under the patient instruction of Julia Troy. The studies showed that, as in humans with early diabetes mellitus, rats with moderate hyperglycemia developed heightened filtration driven by increased glomerular plasma flow and capillary pressure.

Within the department of pathology, there was a vibrant, independent interest in experimental renal disease and pathophysiology. This emphasis on investigation beyond straightforward morphologic descriptions was attributable in large measure to the late Ramzi Cotran, then chair of the department. Manjeri Venkatachalam and Helmut Rennke, both protégés of Cotran, were actively investigating glomerular permeability to macromolecules but using protein probes such as horseradish peroxidase rather than dextrans. In addition to using clearance techniques to pursue the problem, they naturally were interested in the structural correlates to the defects. They had become intrigued with the possibility that at extremes of glomerular hemodynamics, more linear probes such dextrans might uncoil like a snake and travel through the glomerular barrier in a reptilian manner. Having examined papers that dealt with the remnant kidney model, they were aware that this model developed proteinuria in conjunction with severe structural changes. They speculated that perhaps differences in permeability might be detected depending on whether one used a dextran or a protein marker. At that time, the state of knowledge also included the understanding that single-nephron GFR (SNGFR) and plasma flows increased in residual nephrons and that these compensatory changes were inversely related to the degree of renal mass removed. Indeed, several years before, Barry Brenner, Bill Deen, Dave Maddox, and Channing Robertson had found that modest increases in glomerular pressure also contributed to the increased SNGFR after unilateral nephrectomy. Because the remnant kidney model developed arterial hypertension, Venkatachalam and Rennke were taken with the idea that it might be a situation in which “reptation” could be observed *in vivo*.

In meetings between the two laboratory groups, Rennke and Venkatachalam suggested that Jean Olson, who was a fellow from Johns Hopkins and about to join their group,

TABLE 1. Summary of renal cortical microcirculation studies in groups I, II, and III

	PUN, mg/100 ml	Hct, vol/ 100 ml	$\overline{AP}$ , mmHg	$P_{i,c}$ , mmHg	$P_{i,v}$ , mmHg	$P_{es}$ , mmHg	$\overline{\Delta P}$ , mmHg	$C_{A, g/}$ 100 ml	$C_{V, g/}$ 100 ml	$\Pi_A$ , mmHg	$\Pi_V$ , mmHg	SNFF	SNGFR, nl/min	GFR, ml/ min	$Q_A$ , ml/ min	$R_A \times 10^{10}$ dyn·s·cm <sup>-5</sup>	$R_{i,v} \times 10^{10}$ dyn·s·cm <sup>-5</sup>	$R_{i,A} \times 10^{10}$ dyn·s·cm <sup>-5</sup>	$K_f$ , nl/(s· mmHg)
Group I (n = 8)	20 ±1	52 1	112 2	49 1	12 1	18 1	37 1	5.1 0.2	8.3 0.4	16 1	34 2	0.38 0.02	27.8 3.2	0.72 0.06	74 11	3.5 0.4	2.2 0.2	6.0 0.6	≥0.041 0.007
Group II (n = 9)	89 ±6	51 2	128 4	63 2	19 1	25 2	44 2	5.3 0.2	8.1 0.3	17 1	34 2	0.35 0.03	62.5 6.4	0.21 0.03	187 20	1.4 0.2	1.1 0.2	2.5 0.3	0.063 0.018
Group III (n = 7)	28 ±6	52 1	117 4	46 1	14 1	19 1	32 3	5.4 0.1	8.7 0.4	17 1	37 3	0.38 0.02	38.2 6.2	0.16 0.01	92 16	2.5 0.5	1.3 0.2	3.9 0.6	≥0.085 0.021
P, I vs. II	<0.001	NS	<0.005	<0.001	<0.001	<0.025	<0.025	NS	NS	NS	NS	NS	<0.005	<0.001	<0.001	<0.005	<0.005	<0.005	NS
P, II vs. III	<0.005	NS	NS	<0.001	<0.025	NS	<0.01	NS	NS	NS	NS	NS	<0.025	<0.001	<0.01	NS	NS	NS	NS
P, I vs. III	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	<0.001	NS	NS	<0.01	<0.05	NS

Values are means ± SE. PUN, plasma urea nitrogen. See glossary for other abbreviations.

could work on the structural and permeability studies. Brenner suggested to Hostetter that he might be interested in pursuing the model from the hemodynamic standpoint as, like diabetes mellitus, it likely would display increased SNGFR.

Venkatachalam prepared some of the first rats for preliminary studies. The infarct model of partial renal ablation was chosen partly because of the studies by Shimimura and Morrison (4), which carefully characterized the model, and by Purkerson *et al.* (5), which examined its responses to several manipulations. Both groups used this infarct approach. Also, the surgical amputation, or "polectomy," model led to such severe scarring and adhesions that subsequent micropuncture was impossible. After the preliminary efforts, rats began to be micropunctured in the third-floor laboratories of the Medical Research Building at the Brigham in the mornings and by the afternoon were ferried across Shattuck Street, still anesthetized, to the pathology laboratory in the Seeley Mudd Building for perfusion fixation of their kidneys.

At the time of submission of the abstract, it was clear that although there was an association among increases in SNGFR, transcapillary pressures, and plasma flows on the one hand and glomerular pathology on the other, a test for causality was needed badly. In fact, before signing the abstract, Brenner exacted a promise that we would devise and conduct a test of the hypothesis. Several ideas were floated, but by chance, at this time in the LKEP, Iekuni Ichikawa and Barry Brenner were collaborating with Saulo Klahr and Mabel Purkerson in studies on the effects of severe reductions in dietary protein on normal renal structure and function. These studies addressed the pathophysiology of protein malnutrition, not progressive renal damage. However, decreased protein intake had received in the previous decades considerable attention as a means of reducing renal injury. Dating back to the 1920s, Newburgh, Addis, Farr, and Smadel were particularly prominent early proponents of decreased protein intake in the United States based on their experiments in animals (3). Giordano, Giovanetti, Walser, and Mitch, among others, had more recently carried the notion to clinical investigation and therapy. We initially saw this dietary approach as a potentially convenient means to test the link between increased hemodynamic forces and injury. Supplied with this background of information to draw upon and with Ichikawa's bag of 6% protein diet to filch from, we set out to test the hemodynamic causation of glomerular injury. The results were dramatic both in magnitude and in consistency. Proteinuria and injury were strikingly attenuated by the low-protein diet, but so were the increases in glomerular pressures and flows. Thus, we had our first proof of the hemodynamic hypothesis. The paper was submitted for publication. To our amazement, it initially was rejected by one journal on "priority grounds" before its final acceptance by another journal. The companion abstract detailing structure and permselectivity was presented by Jean Olson at the meeting of the American Society of Nephrology in the fall of 1979 and later published in full (6).

It is not our intent in this brief reminiscence to analyze in detail the intellectual currents that buoyed this work. Surely, the concepts and techniques of glomerular physiology and morphology, a recognized model and a potent manipulation, all were key elements. However, the older micropuncture studies of the late Carl Gottschalk and his group, which showed wide dispersion of SNGFR in disease, and the balance and

prepared in standard fashion for micropuncture (4) and studied under normal hydropenic conditions. Plasma urea concentration was measured at the beginning of each micropuncture experiment using a Beckman Analyzer 2 (Beckman Instruments, Fullerton, CA). Samples of proximal tubule fluid were obtained by micropuncture for determination of flow rate and inulin concentration, the latter by the method of Vurek and Pegrum (32). Efferent arteriolar blood samples were obtained for measurement of total protein concentration (31). Hydraulic pressure measurements were made in cortical tubules and vessels using the servo-null micropipette technique (4). The details of the calculations employed have previously been given (4). Statistical analyses were performed with the unpaired *t* test. Statistical significance was defined as  $P < 0.05$ .

Following micropuncture, the kidneys from each rat were examined morphologically. For light and electron microscopy, the kidneys were fixed by perfusion at the measured arterial pressure with 1.25% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4); the tissue was rinsed in buffer, postfixed in 1% osmium tetroxide, dehydrated, and embedded in epon 812. Thick sections (1  $\mu\text{m}$ ) were stained with 0.5% toluidine blue in 1% aqueous borax. Thin sections (60–90 nm) were stained with uranyl acetate and lead citrate and examined in a Philips 201 electron microscope at 60 kV. For scanning electron microscopy, tissue after postfixation was dehydrated in graded alcohols, critical point dried with carbon dioxide, and coated with gold-palladium. The tissue was then examined with an Advanced Metals Research 1000A scanning electron microscope at 20 kV. Following dehydration in absolute alcohol, additional tissue was frozen in liquid nitrogen, fractured, and critical point dried. The cleaved surfaces were identified with a dissecting microscope, plated with gold-palladium, and examined with a scanning electron microscope.

## RESULTS

**Micropuncture studies.** Mean values for whole kidney GFR, AP, SNGFR, and the pressures, flows, and resistances governing glomerular ultrafiltration are summarized in Table 1. Mean arterial pressure averaged  $112 \pm 2$  (SE) and  $128 \pm 4$  mmHg in *groups I* and *II*, respectively ( $P < 0.005$ ). Values for whole kidney GFR averaged  $0.72 \pm 0.06$  in *group I* and  $0.21 \pm 0.3$  ml/min in the remnant kidneys of rats in *group II* ( $P < 0.05$ ). In keeping with this reduced GFR, these *group II* rats were azotemic, with a plasma urea nitrogen concentration (PUN) averaging  $89 \pm 6$  mg/100 ml as opposed to  $20 \pm 1$  mg/100 ml in control rats (*group I*) ( $P < 0.001$ ). Values for SNGFR in *group II* animals averaged  $62.5 \pm 6.4$  nl/min, more than twice the mean value of  $27.8 \pm 3.2$  nl/min of *group I* ( $P < 0.005$ ). This marked increment in SNGFR 1 wk after one and five-sixths nephrectomy in *group II* rats could be ascribed mainly to two factors. First, initial glomerular plasma flow rate,  $Q_A$ , was elevated on average to  $187 \pm 20$  nl/min in *group II* compared with  $74 \pm 11$  nl/min in *group I* ( $P < 0.001$ ). Second, the mean glomerular transcapillary hydraulic pressure difference,  $\Delta P$ , averaged  $44 \pm 2$  mmHg in *group II* as compared with  $37 \pm 1$  mmHg in *group I* ( $P < 0.025$ ). This greater average value for  $\Delta P$  resulted despite an increase in proximal tubule hydraulic pressure,  $P_T$ , since mean glomerular capillary hydraulic pressure,  $P_{GC}$ , increased markedly in the remnant glomeruli (Table 1). Systemic plasma protein concentration,  $C_A$ , was not different between these two groups. Thus, the higher average values for  $\Delta P$  and  $Q_A$  were responsible for the augmented driving force for filtration.

clearance studies of Neal Bricker and his colleagues, which expounded the notion of “trade off,” also informed the concepts hopefully advanced by our paper (3).

If the dissection of scientific underpinnings is complex, then assessment of the scientific motives for the work, especially in long retrospect, may be almost impossible. However, this effort did begin in an atmosphere that was charged with interest in very basic issues of glomerular filtration dynamics and capillary permeability to proteins, issues that may have seemed even arcane at the time. Irrespective of its beginnings, we rapidly became aware that the results could have bearing on clinical progressive renal diseases. The discussion section of the paper reflects that growing awareness with its multiple references to clinical observations of others. Furthermore, the work motivated the Brenner laboratory to explore whether a pharmacologic approach also could ameliorate renal injury and eventually led to the recognition that angiotensin-converting enzyme inhibitors are uniquely renoprotective (7). Thus, apart from whatever milestone it may represent, the paper also exemplifies the values of group effort and of physician-scientists engaged in fundamental research.

Brenner and Rennke remain at the Brigham and Women’s Hospital and Harvard Medical School in the Departments of Medicine and Pathology, respectively. Olson is in the Department of Pathology at the University of California in San Francisco. Venkatachalam is in the Department of Pathology at the University of Texas in San Antonio. Hostetter is at the National Institute of Diabetes and Digestive and Kidney Diseases on the National Institutes of Health campus, on leave from the University of Minnesota.

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## GUEST COMMENTARY

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The title of this month’s milestone, “Hyperfiltration in Remnant Nephrons: A Potentially Adverse Response to Renal Ablation,” reflected an important conceptual advance in nephrology. The authors established that increased

The glomerular capillary ultrafiltration coefficient,  $K_f$ , was calculated as its minimal value for *group I* rats because these rats were at or near filtration pressure equilibrium, with no statistical difference between measured values for  $\Pi_E$  and  $\Delta P$  ( $P > 0.2$ ). Unique values for  $K_f$  were calculable for the *group II* animals, however, since they did not achieve filtration pressure equilibrium, with  $\Delta P$  significantly greater than  $\Pi_E$  ( $P < 0.025$ ). Thus, minimal  $K_f$  values averaged  $0.041 \pm 0.007$  nl/(s · mmHg) in *group I*, whereas in remnant glomeruli of *group II* rats unique  $K_f$  values averaged  $0.063 \pm 0.018$  nl/(s · mmHg). Since the *group I* values were minimal, it is not possible to statistically compare them with the numerically greater unique value calculated for *group II*.

Afferent and efferent arteriolar resistances as well as total arteriolar resistances also differed markedly between *group I* and *group II* (Table 1). *Group II* rats had average values for all of these resistances that were half or less of those of *group I* rats. Thus, the elevated glomerular capillary hydraulic pressures found in *group II* rats may be ascribed not only to the higher values of  $\Delta P$  in this group but also to reduced afferent arteriolar resistances,  $R_A$ .

Despite comparable reduction in renal mass, the rats maintained on a low protein diet (*group III*) were found to exhibit strikingly different glomerular hemodynamic patterns from those seen in *group II*. The mean value of SNGFR in *group III* rats ( $38.2 \pm 6.2$  nl/min) was markedly lower than that found in *group II* rats ( $62.5 \pm 6.4$  nl/min) ( $P < 0.025$ ). Indeed, the value in *group III* was not statistically different from that of *group I* ( $P > 0.1$ ). The absence of a sizable increment in SNGFR in *group III* compared to *group I* proved to be due to the failure of  $Q_A$  and  $\Delta P$  to increase in this group to the level seen in *group II*. Once again, these parameters in *group III* were not different from average values obtained in *group I*. Furthermore, the numerically higher minimal mean value of  $K_f$  in *group III*, compared with that in *group I*, would not account for any substantial change in SNGFR in *group III*, since this group, like *group I*, was at or near filtration pressure equilibrium (i.e., no statistical difference between  $\Pi_E$  and  $\Delta P$ ,  $P > 0.2$ ). Thus, low protein feeding in *group III* was associated with SNGFR values in remnant glomeruli with little or no hemodynamic change relative to *group I* animals, despite a degree of ablation equivalent to that of *group II*.

Mean arterial pressure in *group III* averaged  $117 \pm 4$  mmHg, a value roughly midway between those found in *groups I* and *II* and not significantly different statistically from either of them. Also, the average value of total arteriolar resistance,  $R_{TA}$ , in *group III* animals was intermediate between values obtained in *groups I* and *II*, and not significantly different from both. Although the afferent and efferent arteriolar resistances were also intermediate in *group III*, they were not consistently statistically distinguishable from those of *groups I* and *II*.

**Morphological studies.** In each animal, from 10 to 42 glomeruli were examined by light microscopy. Glomeruli from *group II* animals displayed a wide range of morphological abnormalities. Osmophilic droplets representing lysosomes containing reabsorbed protein were present not only in the epithelial cells of glomeruli but also in proximal tubule cells. Attenuation of glomerular epithelial cells resulted in the appearance of blebs, visible by light microscopy in 43% of glomeruli in *group II* rats. Increased mesangium was also discernible in 16% of these glomeruli (Fig. 1A). These alterations appeared to be more frequent in the outer cortex, although juxtamedullary glomeruli were also involved, so that no clear stratification of the lesions could be found. The renal

glomerular capillary pressure and flow were responsible for remnant nephron hyperfiltration, which previously had been identified as a normal response to reduction in nephron number. They then argued that these hemodynamic changes cause remnant glomerular injury. This conclusion was supported by the finding that severe dietary protein restriction, which largely prevented the hemodynamic changes, also largely prevented injury. In broad terms, the authors showed how the kidney, having been injured by external forces, proceeded to further injury itself.

The analysis of self-perpetuating renal injury presented in the report by Hostetter et al. and the companion report by Olson *et al.* (1) stimulated new interest in the experimental study of renal disease progression. This interest continues, as reflected by an increasing number of studies in the remnant kidney and other disease models. Potentially important questions about remnant nephron function, however, remain unanswered. It is hoped that presentation of the report by Hostetter *et al.* as a milestone will motivate further study of these questions. Indeed, reviewing this work should increase our confidence that better understanding of function will lead to better treatment of disease. It is worth emphasizing that the success achieved by Hostetter *et al.* was based on laboriously acquired knowledge of basic renal physiology. Barry Brenner and his associates had developed micropuncture methods and mathematical models that enabled them to quantify the determinants of glomerular filtration. The identification of increased capillary pressure and/or flow as contributors to injury and the subsequent demonstration that angiotensin-converting enzyme inhibition retards the progression of renal disease resulted from the application of these physiologic tools to the remnant nephron (2).

One question raised in the original report has been answered. The authors were careful to note that they could not tell whether injury was due to increased glomerular pressure, flow, filtration, or some combination of these changes. Though they avoided distinguishing increased filtration *per se* as the cause of injury, their work became known as the “hyperfiltration hypothesis,” probably because alliteration made the name easy to say. Subsequent studies established that increased pressure was by itself a major contributor to injury. These studies showed that late institution of protein restriction in remnant kidney rats normalized glomerular pressure and prevented injury without lowering glomerular blood flow or filtration rates (3). Angiotensin-converting enzyme inhibition likewise lowered capillary pressure and limited remnant glomerular injury without reducing remnant kidney perfusion or filtration (2,3). Although these studies indicate that glomerular hypertension is injurious, they should not be considered to show that hyperperfusion and hypertrophy of remnant glomeruli are benign. We have not identified the factors that cause “compensatory” growth and increased perfusion of remnant glomeruli after reduction in nephron number, and we possess very imperfect knowledge of the extent to which interrupting these processes limits glomerular injury.

We also have not identified the cause(s) of systemic and glomerular hypertension after renal ablation. Much new information indeed has been acquired. BP increases after renal ablation in some strains of rats and mice but not in others. BP increases to a different degree when ablation is accomplished by different methods within the same strain.

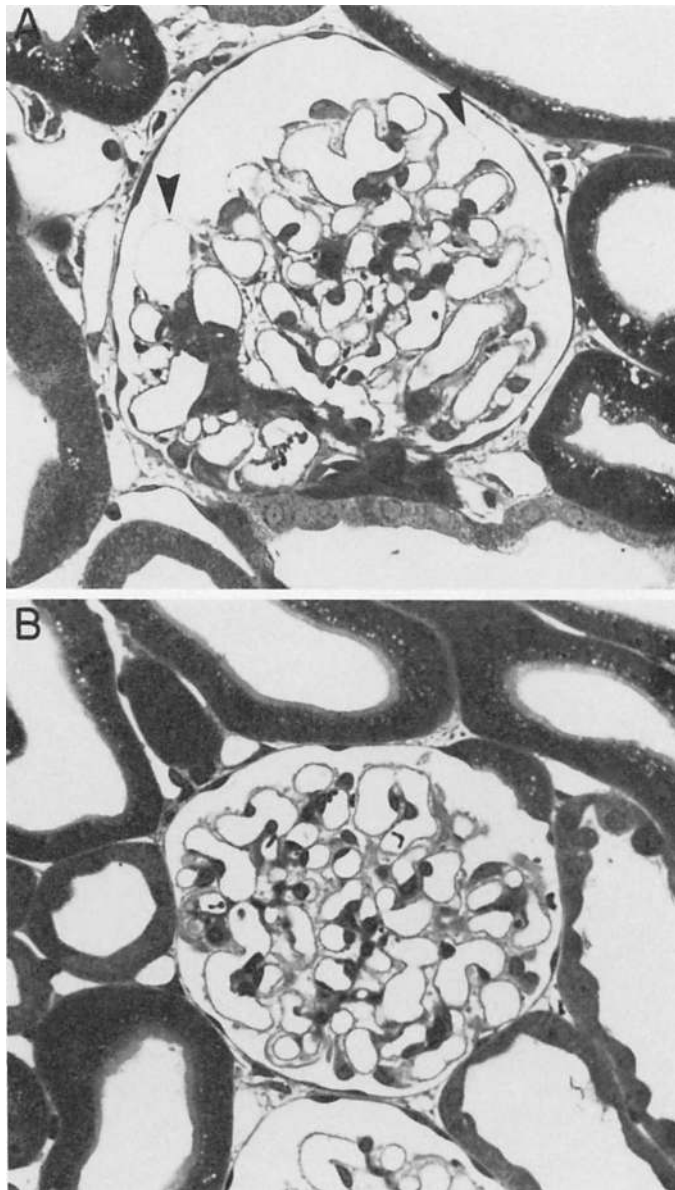


FIG. 1. Light micrographs of glomeruli from experimental rats 7 days following operation. *A*: group II rat. Note segmental increase in mesangium and blebs (arrowheads) in epithelial cells. *B*: group III rat. Glomerulus is normal. (Toluidine blue,  $\times 300$ ).

vasculature was normal, whereas rare hyaline casts were observed in tubules. *Group I* animals demonstrated none of these abnormalities, and in *group III* the extent of the glomerular lesions was markedly diminished (Fig. 1*B*). Though proximal tubule cells in *group III* showed approximately the same amount of protein reabsorption droplets as in *group II*, the number of such droplets in the glomerular epithelial cells was markedly reduced. In addition, fewer than 10% of glomeruli in *group III* demonstrated the blebs of attenuated podocytes and no mesangial enlargement was observable.

Transmission electron microscopy in *group II* demonstrated focal lifting of endothelial cells from the lamina rara interna, with accumulation of flocculent material beneath them (Fig. 2, inset). Occasional endothelial cells of this group contained whorls of membranous material. These whorls also were noted within capillary lumina. Protein reabsorption droplets were present in the epithelial cells of the glomerulus and the proximal convoluted tubule. In the podocytes these droplets were homogeneous but sometimes contained flocculent material.

These differences should provide clues to the genesis of hypertension, but we have not yet solved the mystery. When it develops, hypertension can be reversed by blockade of angiotensin II activity. Neither circulating nor intrarenal angiotensin II activity, however, is increased after renal ablation. Overall, during the 20 yr since the report by Hostetter *et al.*, the problem of how arterial pressure and capillary pressure are controlled in the kidney has come to seem more complex rather than to be solved. Atrial natriuretic peptide, nitric oxide, and endothelin have been added to the list of factors that can alter BP and renal perfusion. Understanding how the factors on this long list interact with each other to determine systemic and renal vascular resistances presents the modern physiologist with a daunting task.

The study by Hostetter *et al.* also raised the question of how hemodynamic changes injure glomerular cells. Studies in vascular biology, a nascent field when this milestone work was performed, have provided possible answers. These studies have shown that the mechanical strain can directly alter vascular cells. The original reports by Hostetter *et al.* and Olson *et al.* (1) described other possible mechanisms of injury, which should not be considered as mutually exclusive with each other or with the more recently identified cellular effects of strain. In particular, these reports relate injury to altered macromolecule fluxes in remnant glomeruli. The quantity of infused tracer macromolecules deposited in the mesangium was increased in remnant glomeruli. It was hypothesized that high capillary pressure and/or flow caused increased delivery of circulating macromolecules into the mesangium, triggering mesangial cell replication and matrix production. Stimulated in part by this observation, mesangial "trafficking" of macromolecules was studied over a number of years, but the issue was largely abandoned before its importance was settled. The reports by Hostetter *et al.* and Olson *et al.* (1) also related glomerular injury to increased passage of macromolecules through the glomerular capillary wall. The focus was on identifying defects in glomerular charge and size selectivity that allowed increased filtration of proteins, manifested by proteinuria. Structural studies, however, also revealed protein uptake by podocytes with increased numbers of pinocytotic vesicles at the base of the pedicels. Despite major advances in podocyte biology, we do not know whether this change represents acceleration of a normal process that is part of the podocyte's maintenance work on the capillary filter. We also do not know whether increased podocyte protein uptake ultimately contributes to podocyte injury.

The studies described in the current milestone report were performed at a very early interval after ablation and focused almost entirely on identifying mechanisms of glomerular injury. Subsequent studies have emphasized that glomerular injury in the remnant kidney and other disease models is invariably accompanied by tubular injury. It is worth asking the same question of the tubules that Hostetter *et al.* asked of the glomerulus. Does reducing nephron number alter tubular function in such a way as to make the tubules wear out faster? Endocytosis of filtered proteins, a normal tubule function that is greatly increased in the setting of glomerular injury, is being actively investigated as a cause of tubular injury. Alterations in remnant tubular metabolism, including increased ammonia production and oxygen consumption, also have been considered as potential contributors to injury.

Similar proteinaceous material was noted in the urinary space. Increased numbers of pinocytotic vesicles were observed at the base of the pedicels. Marked attenuation of the epithelial cell cytoplasm was seen frequently. These attenuations account for the blebs described above by allowing formation of large pockets that communicated with the urinary space. Microvilli and focal foot process obliteration were also noted in podocytes (Fig. 2). The number of mesangial cells and the amount of mesangial matrix were both clearly increased in focal areas, as described elsewhere (22). As with light microscopy, *group I* animals showed no lesions. Transmission electron microscopy in *group III* animals showed some of the abnormalities present in *group II*, although to a lesser extent and involving fewer glomeruli (Fig. 3). Endothelial cells in *group III* rats were unremarkable.

Unfortunately, interest in these latter processes, like interest in the glomerular processing of macromolecules, seems to have declined before firm knowledge of their pathophysiologic importance has been acquired.

What would interested readers of this milestone report have thought if they could have been transported 20 yr into the future and found how far we have come? Surely they would have been elated by the progress in molecular biology and the identification of numerous potential molecular mediators of disease progression. Possibly they might have been a little disappointed not to hear of equally impressive advances in the understanding of remnant nephron function.

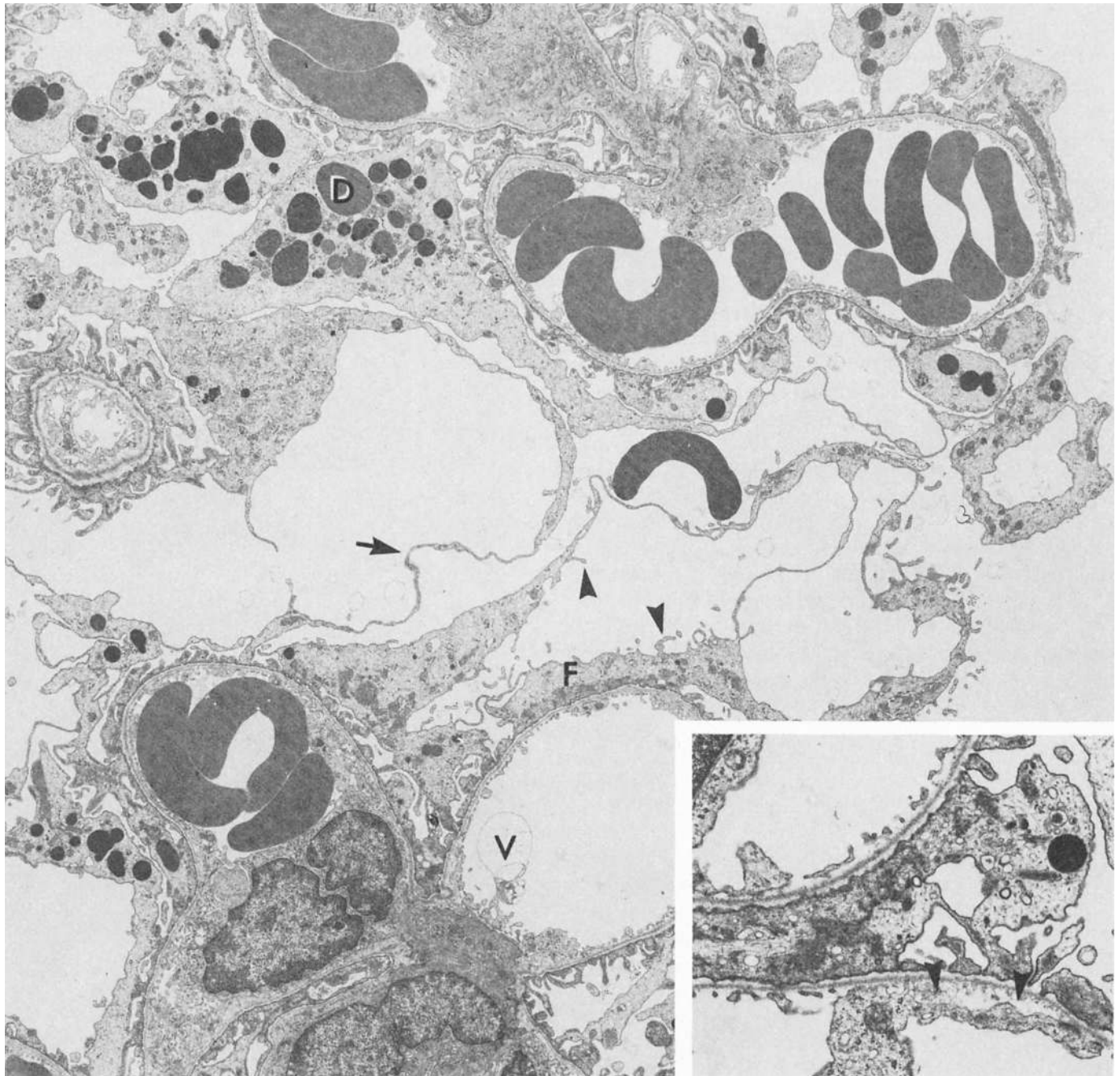


FIG. 2. Transmission electron micrograph of glomerulus from *group II* rat. Vacuolar changes are seen in endothelial cell (V). Protein reabsorption droplets (D), blebs (arrow), microvilli (arrowheads), and focal obliteration of foot

processes (F) are demonstrated. (Uranyl acetate and lead citrate,  $\times 4,050$ .) Inset. Note lifting (arrowheads) of endothelial cell from lamina rara interna. (Uranyl acetate and lead citrate,  $\times 13,350$ .)

Epithelial cells showed no evidence of foot process fusion and somewhat fewer protein reabsorption droplets than in *group II* rats. The mesangium of *group III* animals was not readily distinguishable from that in *group I* controls.

Changes in the morphology of the glomerular epithelial cells of *group II* animals were also well demonstrated by scanning electron microscopy (Fig. 4). Microvilli were very prominent in some areas, and focal obliteration of foot processes contrasted with other areas containing normal interdigitating pedicels (Fig. 4). The blebs were also well demonstrated by this technique (Fig. 4). Fractured specimens were used to examine large expanses of the endothelial surface, as this technique reliably opens glomerular capillaries to view (Fig. 5A). In some areas small blebs were present on the surface of endothelial cells (Fig. 5B), probably corresponding to the collections of membranous and vacuolated material observed by transmission electron microscopy (Fig. 2). In other areas the

Their elation and disappointment might, on reflection, seem to be related. We have found that there is more to do than can be done quickly. The number of potentially important mediators of injury is greater than the number of investigators. Moreover, pursuit of some of these mediators of injury has led investigators away from the study of nephron function. A major effort is being made to profile the expression of cytokines, growth factors, and related molecules in renal disease. It is expected, with good reason, that this knowledge will provide a basis for treatment as means to control the activity of the various molecules are developed. But the alternative investigative strategy of looking for causes of injury in altered function, as originally adopted by Hostetter *et al.*, remains attractive. It offers the appealing prospect that by manipulating function, we can interrupt injury at an early

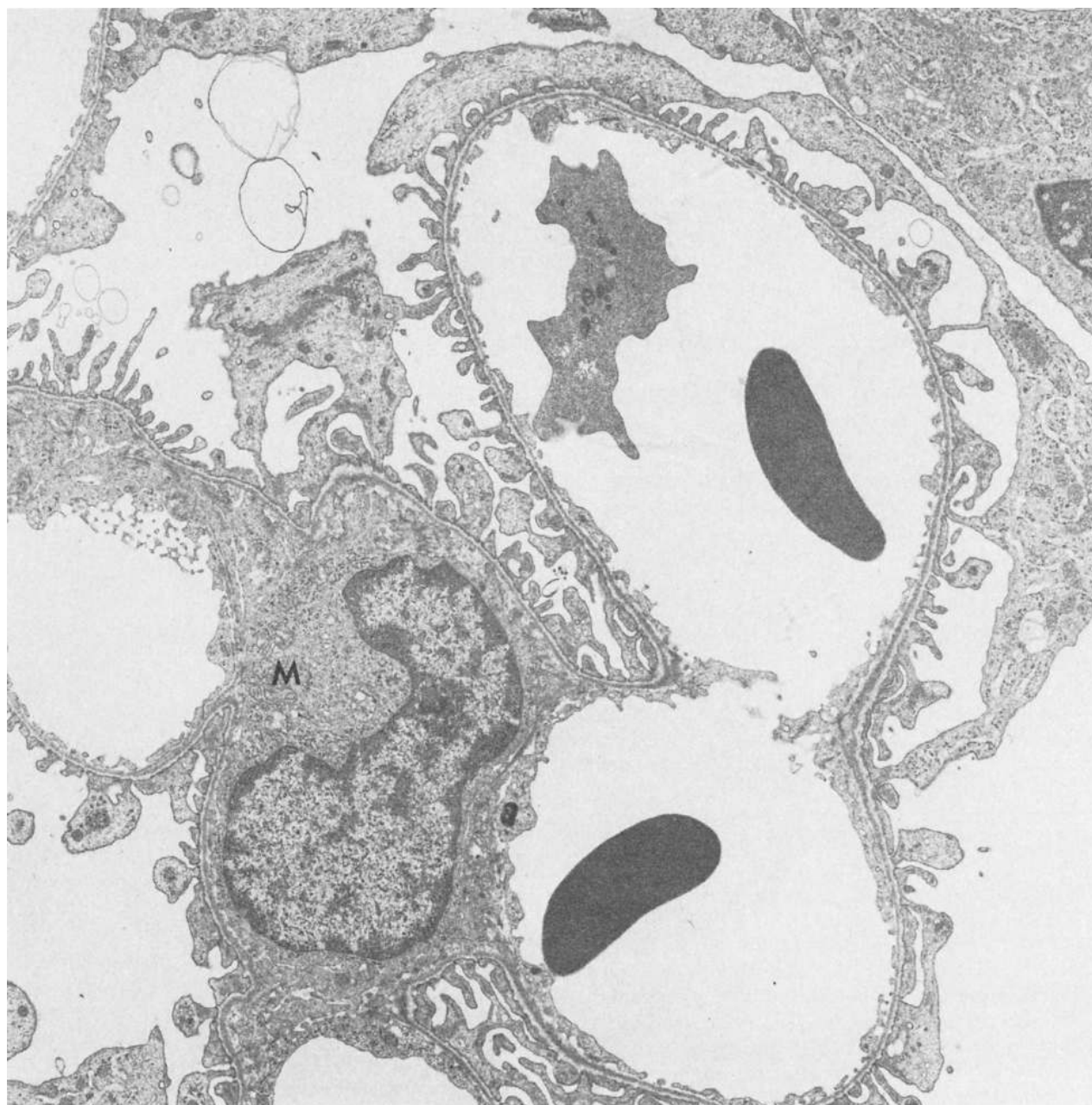


FIG. 3. Transmission electron micrograph of glomerulus from *group III* rat. Note normal mesangium (M) and capillary walls. (Uranyl acetate and lead citrate,  $\times 7,500$ .)



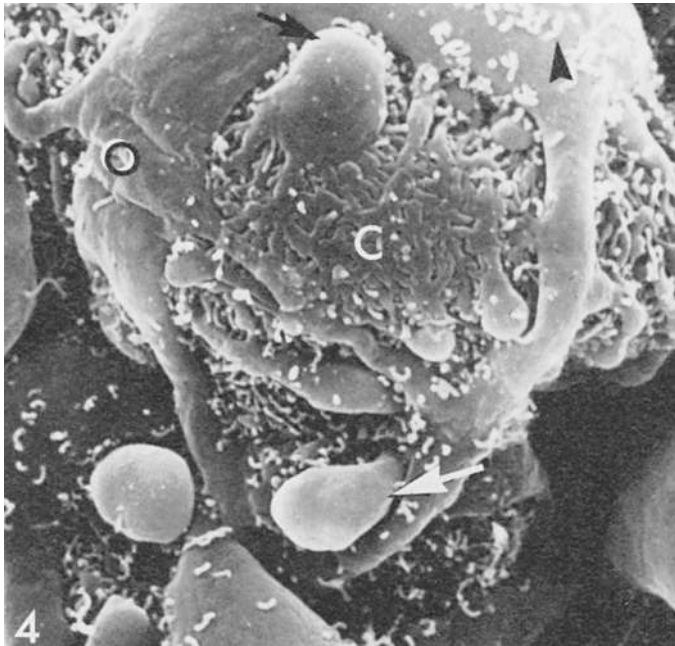


FIG. 4. Scanning electron micrograph of glomerulus from *group II* rat. View from urinary space. Cytoplasmic blebs (arrows), numerous microvilli (arrowhead), focal obliteration (O) and coarsening (C) of foot processes are seen ( $\times 3,600$ ).

endothelium was unremarkable. The glomeruli of both *group I* and *III* animals were entirely normal by scanning electron microscopy.

#### DISCUSSION

The changes in hemodynamics found in remnant glomeruli are generally consistent with those described by other workers studying lesser degrees of reduction in renal mass. Kaufman et al. (17) demonstrated an increase in single glomerular blood flow rate following ablation, as calculated from whole remnant blood flows and glomerular counts, a finding in keeping with the increased  $Q_A$  measured in remnant glomeruli of the *group II* animals of the present study (16). Deen et al. (8), studying glomerular dynamics 3 wk after unilateral nephrectomy, demonstrated an increase in SNGFR of about 80% over values measured in control rats, with proportionally similar increments in  $Q_A$ . Additionally,  $\Delta P$  increased from  $37 \pm 1$  to  $42 \pm 1$  mmHg in that study. These findings were associated with reductions in both afferent and efferent arteriolar resistances (8). Accordingly, the alteration in SNGFR and its hemodynamic determinants were directionally similar in the present study and in the study by Deen et al. (8).

Whether an alteration in  $K_f$  contributed to the observed increment in SNGFR in *group II* is problematic. Since the numerically small average for this parameter was a minimal value in *group I*, it is not possible to compare it with the numerically greater unique value for *group II*. However, it is doubtful that the higher unique value for *group II* animals truly indicates an increase in  $K_f$  above the normal value in this group. In previous studies, unique values of  $K_f$  have been reported for groups of normal animals. These values have ranged from 0.048 to 0.107 nl/(s·mmHg), a range that obviously includes the value calculated in *group II* (21, 29). In addition, the minimal  $K_f$  value calculated in *group III* is not statistically greater than the unique value in *group II*; nevertheless the SNGFR is significantly less in *group III* than in *group II* rats, thus reinforcing the probability that even had

step. It is hoped that the future will see new tools applied to the study of remnant nephron function, resulting in the design of new treatments to slow disease progression.

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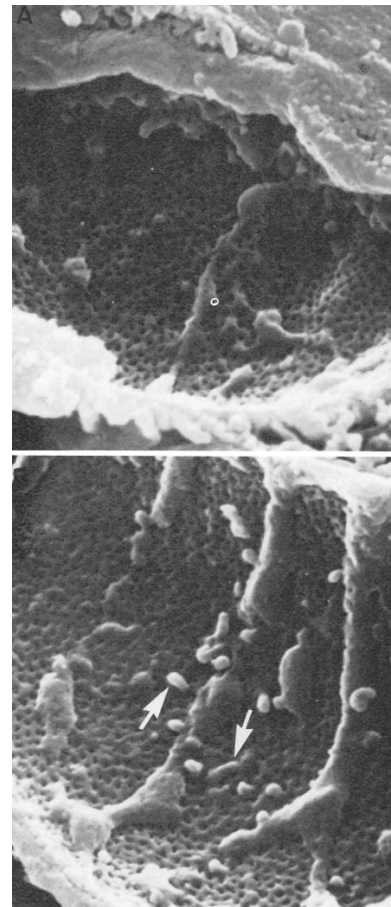


FIG. 5. Scanning electron micrographs of glomerular capillaries. A: normal endothelial appearance is revealed in *group I* rat. B: *group II* rat. Scattered endothelial blebs (arrows) are often present in this group ( $\times 18,000$ ).

some increase in  $K_f$  occurred in *group II* animals, it would not of itself account for the augmented SNGFR in this group (3).

Associated with striking hemodynamic changes, glomeruli of *group II* rats were replete with structural alterations at 1 wk. There were abnormalities of all three glomerular cell types. Endothelial cells showed lifting, membranous whorls, and microvillus formation. Epithelial cell abnormalities were largely those associated with proteinuric states, namely protein droplets, microvilli, and foot process obliteration or “fusion.” Additionally, the epithelial cell blebs, consisting of attenuations of these cells, were noted. These structures have been described in at least two other situations. Elema and Arends (10) observed this abnormality in the glomerular lesion of senile sclerosis of the rat. Also, Takigawa et al. (28) present views of such changes in a study of chronic Masugi nephritis. The pathogenesis of

these blebs is unknown. Last, there is slight mesangial thickening at 1 wk following one and five-sixths nephrectomy.

The possibility of a link between the marked changes in glomerular pressures and flows and these readily apparent structural alterations has been suggested (27). That is, the hyperfiltration (and/or the increased glomerular pressures and flows determining the hyperfiltration) might in itself be the cause of the altered glomerular morphology (27). To test this hypothesis, we studied rats fed a low protein diet. This maneuver was employed in an attempt to vitiate the hyperfiltration of remnant glomeruli observed with ablation in *group II* rats fed a normal diet. We used this dietary manipulation based on the observations that high protein feeding leads to increased renal hypertrophy after unilateral nephrectomy and that general dietary restriction blunts this process (19, 33). Furthermore, studies by Ichikawa et al. (15) demonstrated that rats fed this diet from soon after weaning to age 5 mo had a mean SNGFR 35% less than rats pair-fed a high protein diet. This method of influencing renal function proved to influence markedly the hemodynamics of the residual nephrons. The average SNGFR value, although numerically slightly higher than in *group I* rats, was not statistically greater: indeed, this average value was significantly lower than that in *group II* rats. Furthermore, the structural changes seen in association with single nephron hyperfiltration in *group II* were strikingly reduced in *group III* rats given this low protein diet. Nevertheless, near normalization of SNGFR and its hemodynamic determinants relative to *group I* was accompanied by remarkable amelioration of the structural abnormalities seen in remnant glomeruli of *group II* animals with absolute single nephron hyperfiltration.

These studies are consistent with the possibility that single nephron hyperfiltration (and/or some hemodynamic determinant thereof) is responsible for the early morphological derangements observed in *group II* rats. However, it is possible that some effect of the low protein feeding other than one relating to glomerular hemodynamics accounted for the lesser glomerular damage in *group III* rats. Several recent studies have suggested that phosphorus restriction has a beneficial effect on the progressive uremia seen in rats with marked degrees of renal injury (14, 16). We doubt that changes in dietary phosphate account for the difference between *groups II* and *III* observed in the present study. Dietary phosphorus content was comparable in the normal and low protein diets, being 0.99 and 0.70%, respectively, both well above the 0.04% level used in the studies of phosphorus restriction cited above. More important, no calcium or phosphorus deposits were demonstrable in the remnant kidneys at this early stage of the hypertrophy process.

If hemodynamic alterations underlie this glomerulopathy, the question arises as to how these alterations exert their effects. The mechanism whereby hyperfiltration and/or its hemodynamic determinants alter glomerular structures is not known; however, several possibilities present themselves. The increased glomerular transcapillary hydraulic pressure difference,  $\Delta P$ , may injure the capillary network by some mechanism analogous to the effects of hypertension on the systemic arterial vessels, presumably involving mechanical disruption of normal vascular integrity (23). In this regard, the increased systemic arterial pressure demonstrated by the animals in *group II* could have contributed to the observed glomerular abnormalities. However, it seems unlikely that systemic hypertension adequately accounts for the functional and structural changes observed. For example, Purkerson et al. (24) reported that rats subjected to only partial infarction of one kidney developed arterial hypertension, with a mean arterial pressure of 153

mmHg. But in spite of this hypertension, these rats, with only a minor portion of the renal mass removed, did not exhibit structural changes in their glomeruli (24). These investigators also were able to reduce systemic blood pressure to near normal with antihypertensive drugs in other animals subjected to a degree of renal ablation comparable to that employed in the present study. Nevertheless, a significant degree of glomerular damage ensued. These various findings prompted Purkerson et al. (24) to suggest that uremia or a critical reduction in renal mass was necessary for the progressive glomerular lesion. In addition, the pattern of intrarenal resistances, and consequently glomerular pressures and plasma flows, observed in *group II* animals was quite different from that described in several other models of chronic hypertension in animals with no reduction in renal mass (1, 9). These other hypertensive models have displayed no increase in glomerular capillary hydraulic pressures despite systemic hypertension and have demonstrated reductions in glomerular plasma flow rate. Both of these findings were associated with increased intrarenal resistances (1, 9). In contrast, the rats in *group II* had reduced afferent and efferent arteriolar resistances in the face of mild systemic hypertension, and, therefore, had increased glomerular capillary hydraulic pressures and plasma flows. It is interesting to speculate that this failure to autoregulate glomerular capillary hydraulic pressure and plasma flow may underlie the hemodynamic abnormality of pathogenetic importance in this model. In this regard, Azar et al. (2) have suggested that reductions in intrarenal resistances may play a key role in the glomerular damage seen with unilateral nephrectomy in the so-called "post-salt" hypertensive model. Also, Feld and co-workers (11) have speculated that a relative inability of the deep cortical nephrons to autoregulate was somehow responsible for the greater glomerular injury that this nephron population suffers in the spontaneously hypertensive rat. In any case, it seems reasonable to conclude that the changes in glomerular dynamics and structure that we observed in *group II* rats were probably not a simple consequence of systemic arterial hypertension.

The increased movement of filtrate across the glomerular capillary wall in *group II* rats would be expected to entail an increased transcapillary convective flux of macromolecules as well as water and crystalloid (7). This increased transglomerular traffic of plasma proteins may ultimately have had an injurious effect on some component or components of the glomerular capillary wall (5, 6, 12, 30). In addition, changes in the intrinsic permeability of the glomerular wall might have occurred and resulted in increased movement of macromolecules through the wall, thereby contributing to the ultimate injury of remnant glomeruli (12, 30). Indeed, proteinuria does occur within 1 wk of renal surgical ablation in this model. The potential significance of this finding is discussed elsewhere (22).

Clinically, chronic renal insufficiency regularly progresses to end-stage renal failure, and several studies indicate that for any given patient the rate of progression is predictable (18, 25). Interestingly, these studies indicate that the predictable rate of decline in renal function is idiosyncratic, varying from patient to patient, and apparently not related to the underlying cause of the chronic renal insufficiency (25). It seems quite plausible that after some critical reduction in nephron mass by an initial disease process, a progressive destruction of remnant glomeruli might proceed in remnant nephrons by one or more final common pathways. For example, evidence has recently accrued demonstrating that calcium deposition in the renal parenchyma may represent one such pathway (14, 16). The present studies indicate that intrarenal hemodynamic

derangements may be another common pathogenetic mechanism leading to renal failure after any of a variety of initial insults have reduce nephron mass below some critical level.

In summary, 1 wk after ablation of approximately eleven-twelfths of the renal mass in the rat, remnant glomeruli exhibit striking structural abnormalities in association with a marked increase in SNGFR, the latter due to greatly augmented glomerular pressures and flows. The level of single nephron hyperfiltration can be largely prevented by a low protein diet, and this restraint on the filtration rate despite loss of renal mass is accompanied by near normal values of glomerular pressures and flows. Under these circumstances, the glomerular structural lesions are also largely prevented. These findings, therefore, suggest that "adaptive" increments in SNGFR may represent a potentially adverse response to severe reduction in functioning renal mass and contribute to the progressive destruction of remaining glomeruli that occurs in humans and animals. The

role of these potentially adverse adaptations in other forms of progressive renal insufficiency remains to be determined. However, the possibility exists that such adverse adaptations may constitute a common path for the final destruction of remnant nephrons after any of a variety of initial injuries have reduced renal mass below some critical level.

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