MORPHOMETRY OF THE RENAL CORPUSCLE DURING NORMAL POSTNATAL GROWTH AND COMPENSATORY HYPERTROPHY

A Light Microscope Study

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ABSTRACT

Renal corpuscles from the juxtamedullary and subcapsular regions of the renal cortex were morphometrically analyzed in young rats and in adult rats that had been unilaterally nephrectomized or sham-operated at an early age. Mean corpuscular volumes increased 4.5-fold during normal development, and 7.7-fold as a result of compensatory hypertrophy in both cortical regions. Relative and absolute volumes were determined for Bowman's space, the glomerular tuft, and five glomerular components: epithelial, endothelial, and mesangial cells, capillaries, and the filtration membrane. Normal and hypertrophic enlargement of Bowman's space was slightly greater than glomerular growth, and the growth response of subcapsular glomeruli was greater than that of juxtamedullary glomeruli. The ratio of mean glomerular volumes between outer and inner glomeruli was 1:2 in both adult groups. Both adult groups also developed nearly identical proportions of all glomerular component structures, representing a relative decrease of epithelial cells and increase of capillaries compared to the young animals. Normal and hypertrophic maturation involved absolute increases in all glomerular cell populations, the length of capillary loops and the surface area of the filtration membrane, all nearly in proportion to the respective four- and seven-fold increases in glomerular volume. Changes in the filtration surface area are consistent with published data for glomerular filtration rates in normal and hypertrophied kidneys. The mean cell size in epithelial and mesangial populations doubled during growth, but was not greater than normal in mononephrectomized rats. Hyperplasia among all populations of glomerular cells is indicated in normal growth, and to a greater extent in compensatory renal hypertrophy.

KEY WORDS morphometry · renal corpuscle · normal growth · compensatory hypertrophy.

The kidney has a remarkable capacity to enlarge its mass in response to an increased work load (19, 24, 28). This adaptive growth occurs with a rapid

increase in the size of individual nephrons, resulting from both hypertrophy and hyperplasia of preexisting cellular components of the tubular epithelium (14, 27). However, cellular hypertrophy represents the dominant mechanism (14, 19, 27).

Although changes in tubular structure and function accompanying compensatory hypertrophy have been studied extensively, considerably less information is available concerning the morphological adaptability of the different components in the renal corpuscle. On a functional basis, the glomerular filtration rate rises as well as tubular reabsorption, maintaining the glomerular tubular balance (2, 8, 16, 24). It is reasonable to assume that the increased filtration capacity of the renal corpuscles is paralleled by structural changes inside the glomerular tuft. The absence of mitotic figures in the cellular populations of the renal corpuscle during normal growth (9) and in compensatory hypertrophy (25), both conditions in which the functional demands are enhanced, suggests that any increase in size is the result of the development of larger cells. However, glomerular cells possess the ability to proliferate, as demonstrated by quantitative methods of analysis in some renal diseases (11, 17, 18).

The present study is a morphometric investigation at the level of light microscopy undertaken to measure changes in the structure and cellular composition of renal corpuscles during postnatal development and compensatory hypertrophy. The juxtamedullary and subcapsular renal corpuscles have been separately analyzed in view of the functional differences between glomeruli localized in these two regions of the renal cortex (13).

MATERIALS AND METHODS

12 male Wistar strain rats weighing 35–45 g were fed a commercial pellet diet with water ad lib., and divided into three groups of four animals each. All animals were anesthetized with intramuscular injections of Nembutal (sodium pentobarbital, Abbott Laboratories, North Chicago, Ill., [4 mg/100 g body weight]). The right kidney of each animal in the first group, representing young rats, was immediately fixed by perfusion and prepared for microscope examination. Compensatory hypertrophy of the right kidney was produced in rats of the second group by removal of the opposite kidney. The left kidney was approached through a dorsal subcostal incision. After careful dissection, the hilum was tied off with a single ligature, and the kidney was removed with minimal bleeding. The third group of young rats was subjected to a sham operation. All rats in the second and third groups, representing mononephrectomized and control adult animals, respectively, were sacrificed 35 days after the operation, at which time their right kidneys were fixed by perfusion.

The perfusion fixation was done by first isolating the abdominal aorta and ligating the mesenteric and iliac arteries. A polyethylene cannula (0.5 or 1.5 mm in diameter), filled with pH 7.2 phosphate buffer containing 100 IU heparin per ml, was then inserted into the aorta cephalad to the iliac arteries, sealed in place by means of a ligature and attached to a pressure controlled perfusion apparatus (1). The tip of the cannula pointed in a retrograde direction and was located just below the level of the left renal vein. After measuring the aortic pressure with the perfusion apparatus, the thoracic aorta was clamped, an opening cut in the vena cava, and the kidney perfused for 90 s with the heparinized buffer, followed by a 10-min perfusion with a formaldehyde-glutaraldehyde fixative (15), both at the previously recorded aortic pressure.

After completion of the perfusion, the right kidney of each animal was excised, weighed, and cut into 1-mm wide strips perpendicular to the long axis and through the full thickness of the cortex and medulla. Under a dissecting microscope, 25 pieces of tissue were obtained, which included the cortex and outer medulla. The specimens were kept an additional 3 h in fresh fixative at room temperature, washed several times with 0.1 M phosphate buffer (pH 7.2), and stored overnight at 4°C. They were then postfixed in phosphate-buffered 1% osmium tetroxide, dehydrated with acetone, and embedded in Araldite.

Three blocks were chosen at random from this primary sample from each animal. Sections containing the full thickness of the cortex and outer medulla were cut at 0.5 μm with a Reichert OmU-2 ultramicrotome (American Optical Corp., Scientific Instrument Div., Buffalo, N. Y.), and stained with methylene blue and safranin for morphometric analysis. Of the renal corpuscles present in the sections, only those localized in the uppermost layer of the cortex (subcapsular) and those below the arcuate vessels (juxtamedullary) were considered in this study.

A random sampling of 75 juxtamedullary and 75 subcapsular renal corpuscle profiles was examined in each rat, making a composite sample of 300 corpuscles in each cortical location from each of the three groups of four animals. The maximum and minimum diameters, measured between the inner edges of the thin parietal layer of cells forming Bowman's capsule, were determined on the screen of a Reichert Visopan microscope at a magnification of 770 × for each successively encountered corpuscular profile, regardless of its size. Because the ratio of these diameters was close to unity (mean values all < 1.045), and well below the limit of 1.4, proposed as the highest value that allows the treatment of ellipsoids as spheres (32), the geometric mean of the major and minor axis measurements was considered to be the diameter of each renal corpuscle profile. These

data were divided into size classes of a 10-μm width, and used to reconstruct the real distribution of renal corpuscle diameters by means of a Wicksell transformation (31), using the procedure of Schwartz-Saltykov (29) applied to each group of 300 measured corpuscles. The mean diameter and SEM (standard error of the mean, n = 300) was calculated from each distribution. The corresponding mean ± SEM volumes of the six categories of renal corpuscles was then determined on the basis of a spherical shape.

The partial volumes occupied by Bowman's space and the glomerular tuft within renal corpuscles were morphometrically determined (20, 30) by superimposing a 10 cm-square grid, containing 100 sampling points, over the entire image of randomly selected renal corpuscular profiles. For the group of young rats, a magnification of 1,200 was utilized so that each sampling point represented a tissue area of 69.4 μm^2. A magnification of 490 was used for the sections of adult control and mononephrectomized rats, resulting in a sampling area of 416 μm^2 per point. The number of points overlying Bowman's space and glomerular tuft structures were counted as a measure of the volume fractions of these components within the renal corpuscle. A total of 300 juxtamedullary and 300 subcapsular corpuscle profiles were analyzed this way in each group of animals. The mean volumes of Bowman's space and the glomerulus per corpuscle were determined by multiplying their respective volume fractions by the previously calculated mean corpuscular volume.

Component structures of the glomerulus were measured in a similar manner, using 20 micrographs, five random glomerular profiles taken from each animal, for each of the six classes of glomeruli. Micrographs were printed at × 1,900 and × 1,200 for the young and adult rats, respectively, and a square array of 437 sampling points (corresponding to 25.87 and 64.87 μm^2 per point) was used to cover each glomerular profile completely. Different magnifications were employed because of the larger average size of adult glomeruli; however, the variation in magnification did not affect the resolution of the structures analyzed. Point counts, measuring the absolute area and volume fraction of subglomerular structures, were determined for those overlying epithelial, endothelial, and mesangial cells, and those overlying the filtration membrane and capillary lumina. The epithelial cell fraction, or podocyte fraction, was composed of the nucleus, cytoplasm, and foot processes. The endothelial cell component included the nucleus and the perinuclear cytoplasm, whereas the attenuated cytoplasmic fraction, perforated by the characteristic fenestrae, was combined with the basement membrane. The mesangial fraction included the individual cells, the matrix, and the axial portion of the capillary basement membrane. The filtration membrane fraction was composed of the peripheral basement membrane, the adjacent internal surface of the foot processes, and the attenuated cytoplasm of the endothelial cells. The capillary lumen component was represented by the empty spaces inside the capillaries. The absolute mean volume of each of these components was determined from the product of its volume fraction and the mean glomerular volume.

The same 120 micrographs were also used to estimate the cellular and capillary density within glomeruli and the surface area of the filtration membrane. The cellular density was determined by counting the numbers of individual epithelial, endothelial, and mesangial cell nuclei in each micrograph. These counts were then divided by their respective cellular areas determined from the point-counting procedure described above. The number of transections of capillaries per unit area of glomerulus was counted and multiplied by 2 to yield the length of capillary loops per unit of glomerular volume (6). Dividing the luminal volume of capillaries by their length then gave the mean cross-sectional area of glomerular capillaries perpendicular to their long axis. This figure is also equal to one-half the average area of capillary luminal profiles. The filtration membrane surface was measured with a separate grid consisting of 21 line segments within a circumscribed area smaller than the glomerular profiles. This grid was randomly positioned over each glomerulus, and the number of intersections occurring between the sampling line and the filtration membrane was recorded. The length of sampling line per micrograph corresponded to 132.6 μm at × 1,900, and 210 μm at × 1,200. The surface area per unit volume (square micrometers per cubic micrometers) is equal to twice the number of intersections per micrometer of sampling line (20).

RESULTS

The fixation procedure accomplished a uniform perfusion of the vascular network of glomeruli, characterized by open and empty capillary lumina surrounded by easily recognizable cellular components (Figs. 1–3). The structure of the renal corpuscles in the two types of adaptive growth examined revealed no gross alterations. The distribution of capillaries and cells did not differ from that observed in young animals (Fig. 1), other than the visually evident increase in Bowman's space associated with the presence of a decreased density of nuclei over comparable glomerular areas (Figs. 2, 3). This latter change is the result of proliferation of capillaries which are homogeneously distributed inside the glomerular tuft (Figs. 2, 3). No mitotic activity was seen in the cellular elements of the glomeruli.

Table I shows the mean body weights of the three groups of rats and the mean weights of their right kidneys. The sixfold growth from young to adult animals was not impaired by mononephrectomy, and was nearly paralleled by the increase in

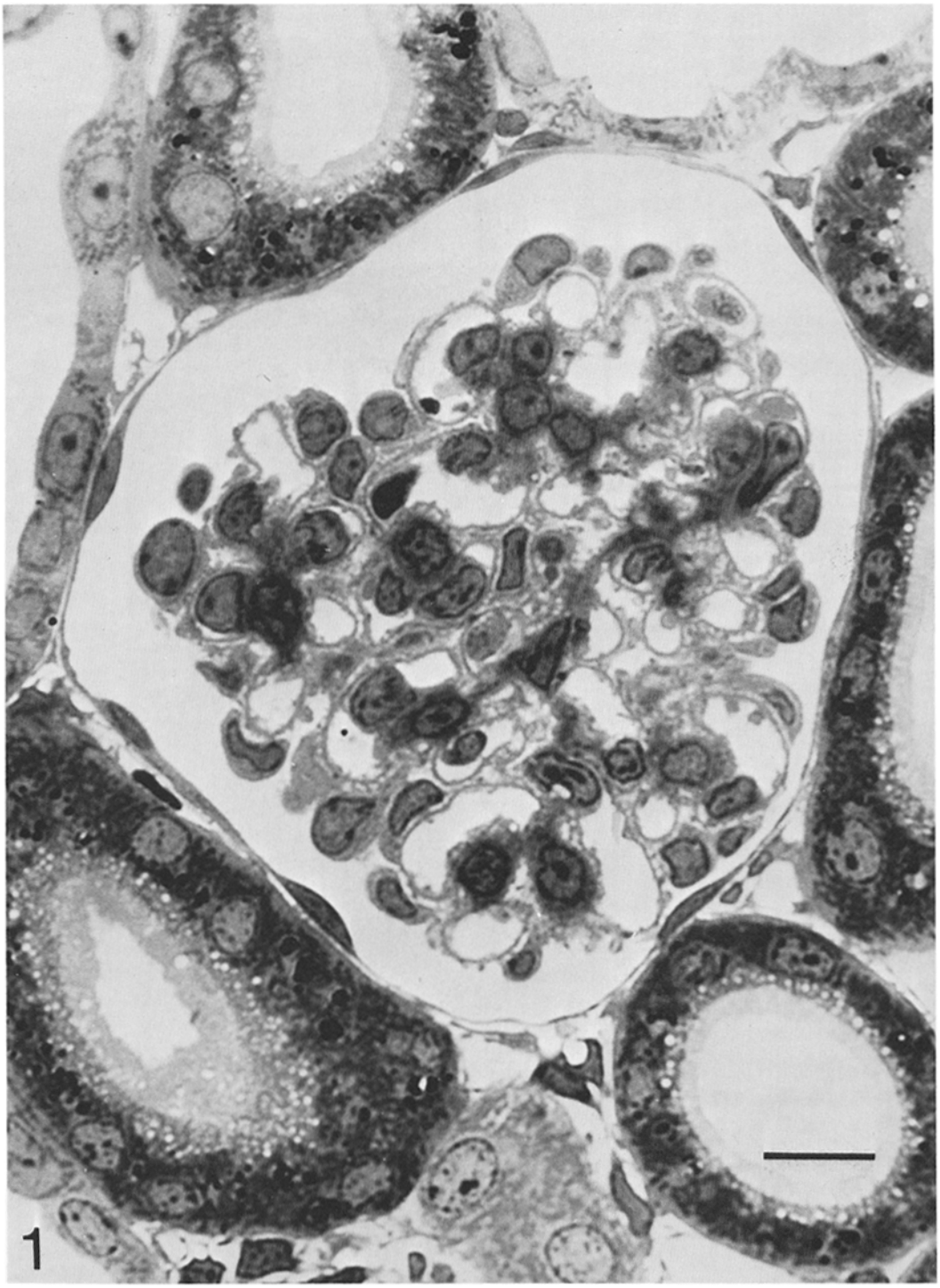

FIGURE 1-3 Examples of glomeruli distributed in the juxtamedullary region of young (Fig. 1), adult (Fig. 2), and hypertrophic kidney (Fig. 3). The vascular network of the glomerular tuft is composed of empty capillary lumina delimited by relatively smooth endothelial profiles. Magnifications are slightly smaller than those used for the morphometric analysis. Bar, 10 μm. Fig. 1, × 1,640; Figs. 2 and 3, × 1,000.

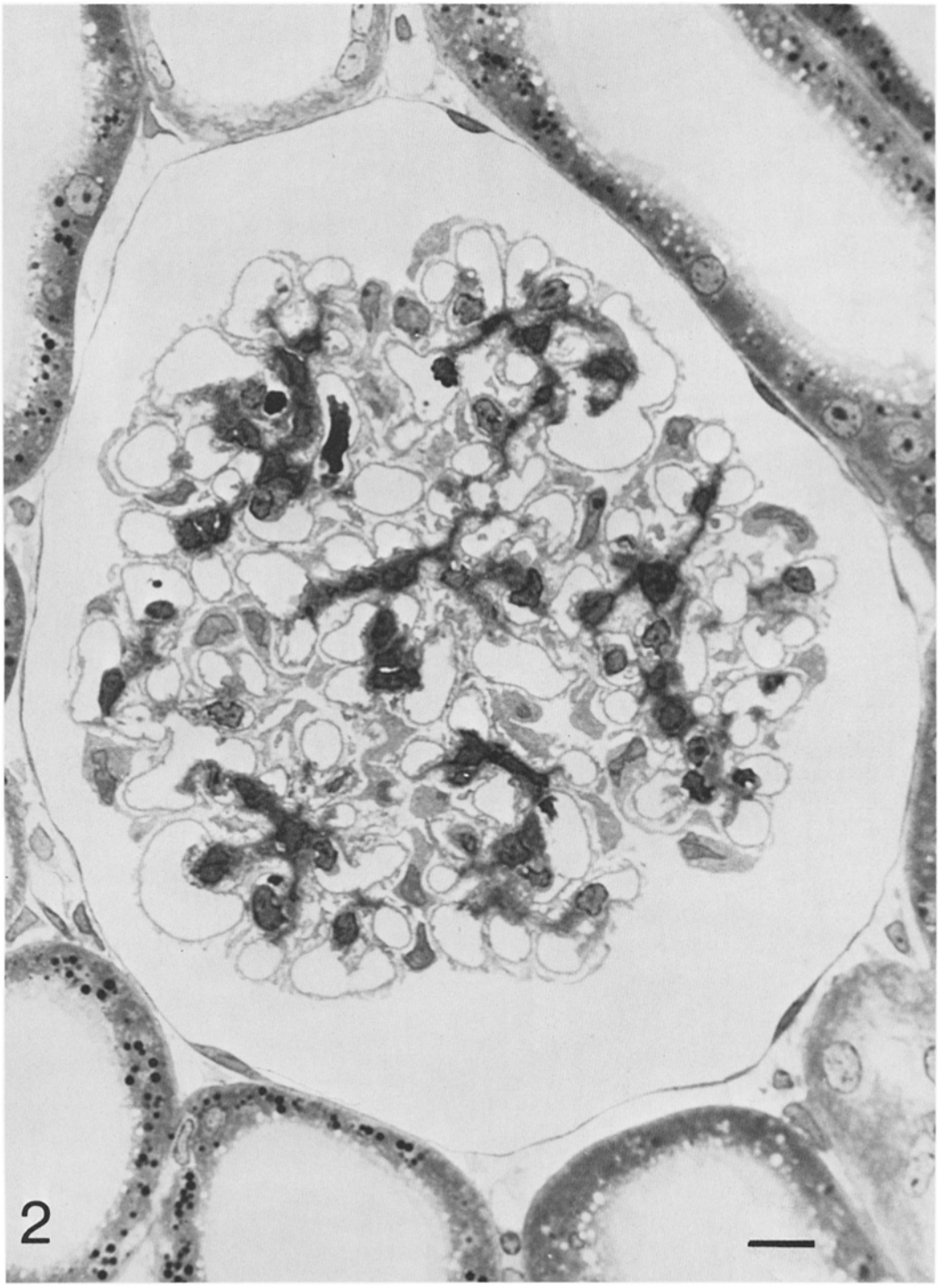

FIGURE 2

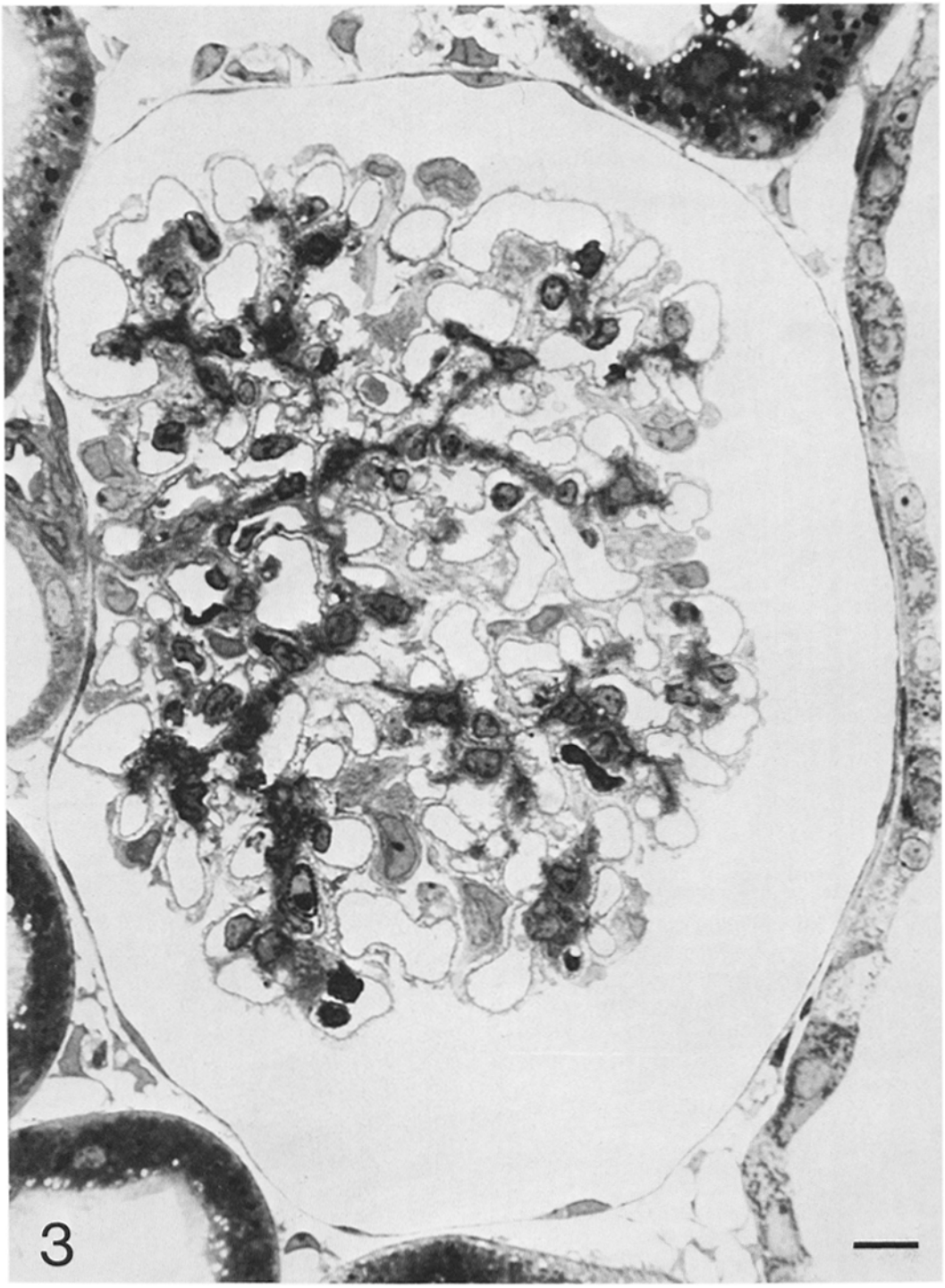

FIGURE 3

TABLE I
Growth Changes in Control and Mononephrectomized Rats

		Young	Adult	Mononephrectomized
Number of animals		4	4	4
Rat weight, *g*		41.8 ± 2.1*	250.8 ± 4.9 (6.00)‡	247.3 ± 5.4 (5.92)
Right kidney weight, *g*		0.41 ± 0.003	1.90 ± 0.13 (4.63)	2.90 ± 0.07 (7.07)
Renal corpuscle		88.83 ± 0.61	146.4 ± 0.68 (1.65)	175.6 ± 0.95 (1.98)
Diameter, μm	JM§	69.17 ± 0.50	114.2 ± 0.63 (1.65)	136.8 ± 0.63 (1.98)
	SC	367 ± 4.4	1,644 ± 13 (4.48)	2,836 ± 27 (7.73)
Volume, $\mu m^3 \times 10^{-3}$	JM	173 ± 2.2	779 ± 7.6 (4.50)	1,341 ± 11 (7.75)
	SC			

* All figures show the mean ± SEM.

‡ Numbers in parentheses represent the factor by which measurements in adult rats, sham-operated and mononephrectomized, are increased in comparison with the group of young animals.

§ Abbreviations JM and SC indicate juxtamedullary and subcapsular regions, respectively.

weight of the right kidney. In comparison with the sham-operated controls, the kidneys remaining after unilateral nephrectomy attained a 53% larger size.

Normal growth and compensatory hypertrophy of the kidney is accompanied by a corresponding enlargement of renal corpuscles in both inner and outer regions of the cortex. Distributions of renal corpuscle diameters were determined for juxtamedullary (Fig. 4 A) and subcapsular (Fig. 4 B) corpuscles in each group of rats, and their mean values are shown in Table I. Juxtamedullary renal corpuscles are uniformly 28% larger in diameter ($P < 0.001$) than those of the subcapsular zone in all groups. After mononephrectomy, corpuscle diameters in the right kidney are 20% greater ($P < 0.001$) than those in the normal adult. The mean volumes of renal corpuscles, which were found to be practically spherical in shape, are also shown in Table I. Juxtamedullary renal corpuscles are 2.1 times larger in volume than subcapsular corpuscles. Normal growth in the sham-operated animals resulted in a 4.5-fold increase in mean corpuscular volume, and compensatory hypertrophy produced a 7.7-fold change and a mean volume 72% larger than normal.

Table II shows the morphometric analysis of the major subdivisions of the renal corpuscle: Bowman's space and the glomerular tuft. The percentage of corpuscular volume occupied by these components was calculated from the tabulated data. The principal change observed is a significant ($P < 0.01$) shift in the volume percent of Bowman's space from ~33% in young animals to 40% in both groups of adults. No other significant differences were found in the relative composition of renal corpuscles, either juxtamedullary vs. subcapsular or sham-operated vs. mononephrectomized adults. The absolute mean volumes of Bowman's space and glomerular tuft per renal corpuscle were derived by multiplying their volume fractions by the mean corpuscular volume given in Table I. All differences between cortical zones and among animal groups are highly significant ($P < 0.001$). The average increase in the volumes of Bowman's space and the glomerulus during normal growth amounted to 5.4- and 4.0-fold, respectively. However, compensatory hypertrophy produced corresponding enlargements of 9.7- and 6.8-fold. Both adult groups showed a somewhat greater growth factor for Bowman's space in juxtamedullary corpuscles and for the glomerulus in subcapsular corpuscles.

Direct morphometric data and results showing the relative composition of glomeruli per unit volume are presented in Table III. No significant differences exist in comparing juxtamedullary and subcapsular glomeruli. Both normal and hypertrophic growth occur with a relative decrease in epithelial cell volume ($P < 0.01$), and a corresponding increase in the volume fraction of capillary lumen ($P < 0.001$). The latter change is accomplished with a decrease in the length of capillary loops per unit volume of larger glomeruli ($P < 0.01$), but a greater increase in their mean cross-sectional area ($P < 0.001$), both alterations being more pronounced in the mononephrectomized group. The mesangial cell population and the surface density of filtration membrane remain essentially constant during age and size changes.

When multiplied by the mean glomerular volumes (Table II), the relative glomerular composi-

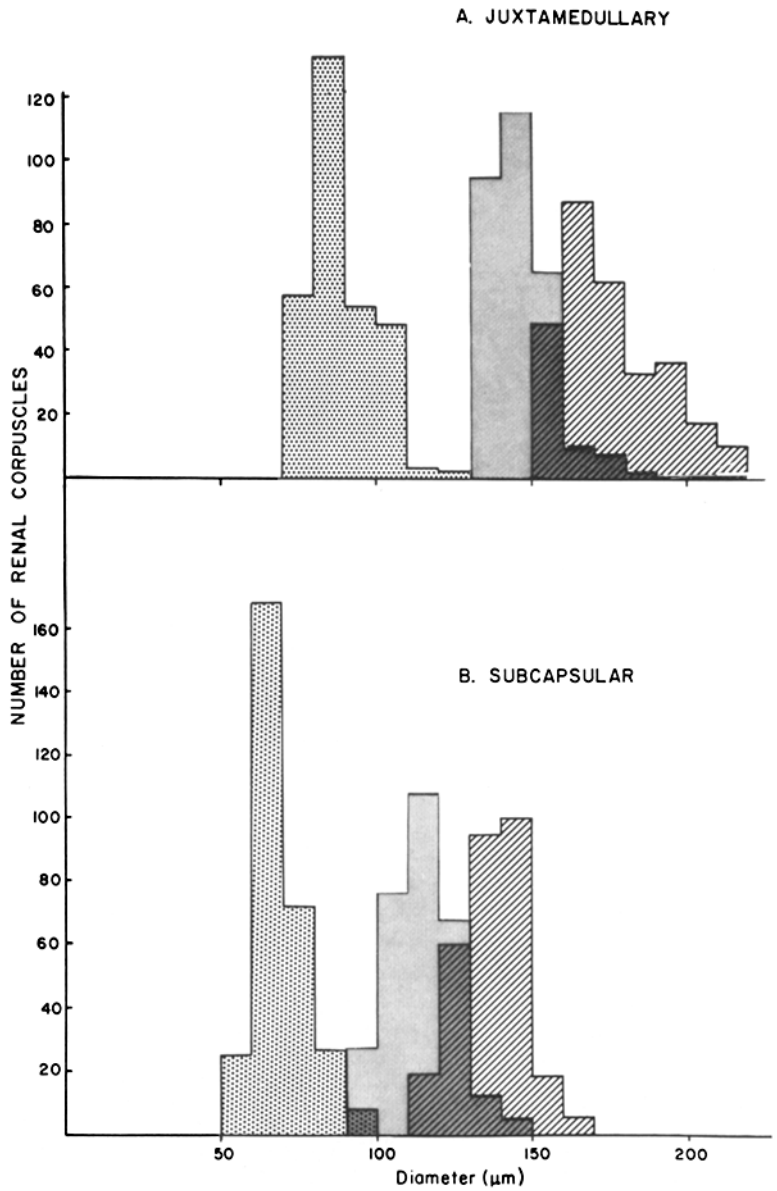

FIGURE 4 Histograms representing the distributions of renal corpuscle diameters in the juxtamedullary (A) and subcapsular (B) regions of the renal cortex. Corpuscle size is smallest in young animals (dotted), intermediate in normal adults (gray), and greatest in the mononephrectomized group (lined). Comparison of top and bottom distributions show the consistently smaller diameter of subcapsular renal corpuscles.

tions (Table III) are translated into the absolute quantities per glomerulus listed in Table IV. Despite the fact that these values contain the cumulative errors from three levels of sampling, corpuscular, glomerular, and subglomerular, practically all differences between juxtamedullary and subcapsular glomeruli and between all three groups of animals are significant at the level of $P < 0.001$. The factors (numbers in parentheses) representing normal growth from young to adult animals indicate a nearly proportional growth of all glomerular components, except for somewhat greater increases in the volumes of mesangial cells and the capillary lumen. The smaller subcapsular glomer-

TABLE II

*Normal and Compensatory Enlargement of Bowman's Space and the Glomerular Tuft**

		Young	Adult	Mononephrectomized
Sampling points counted				
Bowman's space	JM	6,968	4,641	6,462
	SC	4,885	2,857	3,861
Glomerulus	JM	15,331	6,817	8,771
	SC	9,484	4,566	5,957
Volume of renal corpuscle, %				
Bowman's space	JM	31.3 ± 1.3	40.5 ± 0.5	42.4 ± 0.8
	SC	34.0 ± 1.5	38.5 ± 1.0	39.3 ± 0.7
Glomerulus	JM	68.7 ± 1.3	59.5 ± 0.5	57.6 ± 0.8
	SC	66.0 ± 1.5	61.5 ± 1.0	60.7 ± 0.7
Mean volume per renal corpuscle, $\mu m^3 \times 10^{-3}$				
Bowman's space	JM	115 ± 5.1	666 ± 9.8 (5.79)	1,203 ± 26 (10.5)
	SC	49 ± 2.8	300 ± 8.1 (5.08)	527 ± 9.8 (8.93)
Glomerulus	JM	252 ± 5.7	978 ± 11.4 (3.88)	1,633 ± 27 (6.48)
	SC	114 ± 3.0	479 ± 8.9 (4.20)	814 ± 11.0 (7.14)

* See footnotes to Table I.

TABLE III

*Relative Amounts and Growth of Glomerular Components**

		Young	Adult	Mononephrectomized
Area of glomeruli sampled, $\mu m^2 \times 10^{-3}$	JM	68.12	146.3	128.4
	SC	45.74	118.1	106.5
Volume of glomeruli, %				
Epithelial cells	JM	30.6 ± 1.2	24.5 ± 1.2	25.4 ± 1.4
	SC	33.8 ± 2.1	23.3 ± 1.1	24.0 ± 1.9
Endothelial cells	JM	9.5 ± 0.8	7.6 ± 0.6	4.2 ± 0.6
	SC	6.6 ± 0.6	6.3 ± 0.6	5.1 ± 0.5
Mesangial cells	JM	20.0 ± 1.3	23.8 ± 1.4	19.9 ± 1.5
	SC	19.2 ± 1.4	24.7 ± 1.5	21.5 ± 1.8
Capillary lumen	JM	28.8 ± 0.9	36.1 ± 1.4	38.5 ± 1.4
	SC	29.5 ± 1.4	38.2 ± 1.3	39.0 ± 1.7
Filtration membrane	JM	11.1 ± 0.6	8.0 ± 0.6	11.9 ± 1.0
	SC	11.0 ± 0.9	7.5 ± 0.7	10.4 ± 1.1
Capillaries				
Lumina counted	JM	737	1,169	907
	SC	459	966	829
Length per glomeruli, m/mm^3	JM	21.6 ± 1.0	16.0 ± 0.8	14.1 ± 0.8
	SC	20.1 ± 1.3	16.4 ± 0.7	15.6 ± 0.8
Mean cross-sectional area, μm^2	JM	13.3 ± 0.4	22.6 ± 0.9	27.3 ± 1.0
	SC	14.7 ± 0.7	23.4 ± 0.8	25.1 ± 1.1
Filtration membrane				
Intersections counted	JM	257	371	368
	SC	233	384	364
Surface density in glomerulus, $\mu m^2/\mu m^3$	JM	0.194 ± 0.010	0.177 ± 0.010	0.175 ± 0.008
	SC	0.176 ± 0.011	0.183 ± 0.010	0.173 ± 0.011

* See footnotes to Table I.

uli generally show a greater degree of change. The same patterns of growth are also evident in the substantially greater enlargement of glomeruli from animals that have developed a compensatory hypertrophy of the right kidney. In these mononephrectomized rats, the growth factors all range from 1.4 to 1.8 times larger than the corresponding factors for sham-operated controls, except for the volumes of endothelial cells and the filtration membrane. These exceptions probably represent an artifactual inclusion of a greater proportion of attenuated endothelial cell substance within the filtration membrane compartment, as a result of the enlarged capillary size.

Table V shows the differential counts of epithelial, endothelial, and mesangial cell nuclei in glomerular sections. In the lower part of the table, each nuclear count has been divided by the corresponding cross-sectional area of its cell profiles. The resulting numbers are proportional to the

TABLE IV

*Absolute Amounts and Growth of Components**

		Young	Adult	Mononephrectomized
Mean volume per glomerulus, $\mu m^3 \times 10^{-3}$				
Epithelial Cells	JM	77.1 ± 3.5	240 ± 12 (3.11)	415 ± 24 (5.38)
	SC	38.5 ± 2.6	112 ± 6 (2.91)	195 ± 16 (5.06)
Endothelial cells	JM	23.9 ± 2.1	74.3 ± 5.9 (3.11)	68.6 ± 9.9 (2.87)
	SC	7.5 ± 0.7	30.2 ± 2.9 (4.03)	41.5 ± 4.1 (5.53)
Mesangial cells	JM	50.4 ± 4.8	233 ± 14 (4.62)	325 ± 25 (6.45)
	SC	21.9 ± 1.7	118 ± 8 (5.39)	175 ± 15 (7.99)
Capillary lumen	JM	72.6 ± 2.8	353 ± 14 (4.86)	629 ± 25 (8.66)
	SC	33.6 ± 1.8	183 ± 7 (5.45)	317 ± 14 (9.43)
Filtration membrane	JM	28.0 ± 1.6	78.2 ± 5.9 (2.79)	194 ± 17 (6.93)
	SC	12.5 ± 1.1	35.9 ± 3.4 (2.87)	84.7 ± 9.0 (6.78)
Length of capillary, *mm*	JM	5.44 ± 0.28	15.6 ± 0.80 (2.87)	23.0 ± 1.4 (4.23)
	SC	2.29 ± 0.16	7.86 ± 0.37 (3.43)	12.7 ± 0.7 (5.55)
Area of filtration membrane, $\mu m^2 \times 10^{-3}$	JM	48.9 ± 2.8	173 ± 10 (3.54)	286 ± 14 (5.85)
	SC	20.1 ± 1.4	87.7 ± 5.1 (4.36)	141 ± 9 (7.01)

* See footnotes to Table I.

TABLE V

*Differential Nuclear Counts within Glomeruli**

		Young	Adult	Mononephrectomized
Nuclei counted in glomerular areas				
Epithelial cells	JM	389	336	284
	SC	349	297	259
Endothelial cells	JM	319	564	317
	SC	173	368	279
Mesangial cells	JM	398	570	377
	SC	225	486	342
Nuclei per 10^3 μm^2 cell area				
Epithelial cells	JM	18.7 ± 1.0	9.37 ± 0.63	8.71 ± 0.63
	SC	22.6 ± 1.5	10.8 ± 0.54	10.1 ± 0.97
Endothelial cells	JM	49.3 ± 3.7	50.7 ± 3.1	58.8 ± 14.5
	SC	57.3 ± 12.1	49.5 ± 7.9	51.4 ± 7.1
Mesangial cells	JM	29.2 ± 1.7	16.4 ± 0.9	14.8 ± 2.5
	SC	25.6 ± 1.9	16.7 ± 1.3	14.9 ± 1.0

* See footnotes to Table I.

number of nuclei (i.e. cells) per unit volume of cells and, therefore, are inversely proportional to the mean volume per cell. The proportionality factors, from which the absolute mean volumes per cell type could be determined are essentially equal to the mean nuclear diameters in each cell population (20). Although these factors have not been evaluated here, the significant ($P < 0.001$) 50% reductions in nuclear density, observed from young to adult epithelial and mesangial cells, suggest that both of these cell types undergo a twofold hypertrophy during maturation. The remaining cellular growth increments (Table IV) in normal animals, 50% for epithelial cells and 150% for mesangial cells, imply corresponding increases in the numbers of these cells per glomerulus. On the other hand, endothelial cells show no similar indication of hypertrophy, implying that the three- to fourfold increase in their absolute volume per glomerulus is achieved solely by cell multiplication. The nuclear densities per unit cell area in mononephrectomized rats is not significantly different from those of the normal adult. Thus, compensatory hypertrophy of the kidney induced in these experiments produced no additional cellular hypertrophy among glomerular cell populations. However, the enlarged cellular compartments (Table IV) imply that maturation after unilateral nephrectomy of the young rat involves a minimal hyperplasia of all glomerular cells, amounting to more than twofold among epithelial cells, three- to fivefold among endothelial cells, and three- to fourfold among mesangial cells.

DISCUSSION

There are few morphometric studies of glomerular structure, and the quantitative data are difficult to compare because of variations in species, fixation methods, and analytical techniques (4, 6, 7, 23). Perfusion fixation at normal aortic pressure was found here to give excellent preservation of the kidney in both young and adult animals, and to provide a basis for the absolute measurement and comparison of the volume changes occurring in normal growth and compensatory hypertrophy. In contrast with earlier methods in which glomerular size was estimated from its irregular outline (4, 6, 23), the present investigation employed a two-stage sampling procedure. An absolute mean volume of the practically spherical renal corpuscles was determined in the first stage, and in the second stage mean glomerular volume was calculated independently of shape from the fraction of corpuscular volume occupied by glomerular tufts.

Quantitative results show that the average volumes of both glomeruli and whole renal corpuscles in the juxtamedullary region of the renal cortex are more than twice as large as those in the subcapsular region. These relationships, which are seen in postnatal rats as well as in normal and hypertrophied adult kidneys, add further evidence for the morphological and functional heterogeneity of the renal cortex (10, 13, 21, 26). Furthermore, both normal and compensatory growth of the renal corpuscles in each of these populations occurs in identical ratios. The proportion of glomerular growth is slightly less, particularly in the juxtamedullary zone, suggesting a greater adaptability of the more superficial glomeruli.

The use of stained thin sections (0.5 μm) of plastic embedded tissue provides excellent light microscope resolution of the major compartments of tissue structure, and enables the practical application of morphometric (stereological) techniques for measuring their relative volumes and surface areas. The ease of light microscope sampling, in contrast to the use of electron microscopy, is partially offset by its limited resolution of fine detail leading to some systematic errors in the absolute determination of the volumes of certain cellular compartments. Thus, the visceral epithelium in glomeruli, and to a lesser extent the endothelium, will be underestimated because the attenuated regions of these cells, adjacent to the peripheral basement membrane, are included in the composite structure of the filtration membrane. Similarly, the volume of the mesangial cell population is overestimated in proportion to the content of indistinguishable extracellular matrix within the mesangium.

To make an absolute evaluation of cellular hypertrophy and hyperplasia in developing glomeruli, it is necessary to determine the mean volumes and numbers of cells in each population. Morphometric theory shows that the absolute number of nuclei (or cells) cannot be directly obtained from nuclear counts in thin sections, unless the mean nuclear diameters, measured perpendicular to the plane of sectioning, are also known (20, 29, 30). The latter measurement is technically complex for irregularly shaped nuclei. However, even without these data, the highly significant changes observed here in the volumes and nuclear densities of the cellular compartments provide substantial evidence that both hypertrophy and hyperplasia have been demonstrated in normal and compensatory

glomerular growth. A new technique has been recently developed for measuring absolute mean cellular volumes and numbers[1] which, together with electron micrscope morphometry of the glomerulus, should enable a more nearly exact quantitative estimation of these phenomena.

Direct measurements of single nephron glomerular filtration rate (SNGFR) in normal adult and hypertrophic kidney disclose that filtration rate in juxtamedullary glomeruli is always higher than that observed in glomeruli located in the outermost layer of the cortex (13). Soon after birth, the increasing functional capacity of the kidney depends mainly on an increment in the filtration rate of deep nephrons (10, 21, 26). Superficial nephrons make their major contribution only at later periods (21, 26). This centrifugal pattern of development involves both glomerular and tubular components.

Glomerular filtration rate is a function of hemodynamic and oncotic pressures in the glomerular capillaries, glomerular plasma flow, and the filtration surface area and its hydraulic permeability (3). The twofold greater mean surface area of the filtration membrane found in juxtamedullary glomeruli compared with that in superficial glomeruli correlates very well with measurements of corresponding SNGFR (13). A more exact correlation could be achieved only by direct measurements of both factors in a number of individual glomeruli. A simple method enabling the requisite fixation and identification of penetrated nephrons has recently been described for those in the superficial zone of the renal cortex (22). A further agreement between the morphometric results presented here and functional data is seen in the ratio of mean filtration surfaces per glomerulus in subcapsular and juxtamedullary regions. That these ratios are alike, 0.51 and 0.49, respectively for normal and hypertrophied adult kidneys, is consistent with the finding that the normal ratio of superficial to juxtamedullary SNGFR is not changed by hypertrophy after mononephrectomy of either young or adult rats (12).

Normal growth of glomeruli throughout the renal cortex is accomplished by enlarging both the size and number of existing cells, as well as the length and diameter of the capillary loops. After unilateral nephrectomy of the young animals, additional increments in the size of juxtamedullary and subcapsular glomeruli (67 and 70%) principally result from increases in capillary length (47 and 62%) and volume (78 and 73%), and increases in the collective volumes of epithelial cells (73 and 74%) and mesangial cells (40 and 48%). Nuclear count data, with the reservation that nuclear diameters remain constant, indicate no abnormal hypertrophy in glomerular cell populations, implying that cellular volume changes in the enlarged glomeruli are wholly attributable to a proportional hyperplasia. DNA measurements also suggest that a major contribution to cortical compensatory growth in young animals comes from cellular hyperplasia (5). Proliferation of mesangial cells appears to exceed that of epithelial cells during maturation of both normal and overloaded kidneys, however, epithelial cells show a greater differential response. Although no mitotic activity has been observed, these results imply that proliferation of all glomerular cell populations occurs in normal postnatal development, and to a greater extent in compensatory hypertrophy.

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