Intracortical Distribution of Number and Volume of Glomeruli during Postnatal Maturation in the Dog

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ABSTRACT Morphometric analysis was carried out on kidneys of neonatal dogs in which function of the entire kidney and of single nephrons had been evaluated. Measurements were begun after neogenesis of nephrons had been completed, i.e., at the end of the 3rd postnatal wk. They were continued to 74 days by which time glomerular function, expressed per unit of renal weight, had reached the mature level. For statistical analysis, the cortical histogram at each age was divided into eight zones of equal depth between the capsule and corticomedullary junction.

The mean total number of glomeruli in this beagle strain was 589×10^3 per kidney. The fraction of the total number of glomeruli was lowest in the subcapsular layer (3.9%) and highest (24.5%) in the zone immediately beneath from where it decreased almost linearly to a value of 4.5% in the juxtamedullary region. This numerical distribution did not change with age, which suggests that growth of nonglomerular structures proceeded at the same rate in all cortical layers.

Volume of the glomerular tuft rose slightly between the subcapsular and next layer and remained constant down to the juxtamedullary region where it increased sharply. The juxtamedullary glomerulus was about 45% larger in volume than the other glomeruli. This intracortical distribution of glomerular volume did not vary between 23 and 74 days, although the volume of an individual glomerulus at each level increased slightly with age.

Total glomerular volume increased by 33% during the postnatal period studied, whereas simultaneously nonglomerular cortical volume rose by 235%. On the assumption that nonglomerular tissue consists mainly of tubules, the data suggest that the rate of tubular growth far exceeded that of glomerular growth. Despite this difference in glomerular and tubular growth rates, analysis of single nephrons in these dogs demonstrates constant and mature proximal fractional reabsorption of sodium and water.

INTRODUCTION

Development of renal function during early infancy has stimulated attempts to describe structural correlates. The centrifugal pattern of nephron development from the deep cortex toward the periphery (1, 2) suggests that glomerular maturation is not merely a function of age but is also related to the position of the glomerulus at various cortical levels. Maturing human nephrons have been previously examined at three cortical levels by microdissection (3). In the present study on purebred dogs, the number and volume of glomeruli at eight cortical levels and at different postnatal ages were determined by a histological, morphometric method (4). The results indicate that postnatal age of a nephron is linked systematically to its position within the cortex.

METHODS

Dogs of a purebred beagle strain (Omis Beagle Kennels, Roscoe, Ill.) were used. At the end of an acute experiment during which renal function had been evaluated (5), the right kidney was clamped at the hilum, removed, and prepared for histological examination. Kidneys weighing 7.5–15.8 g were obtained from dogs at 23, 40, 48, 60, and 74 days after birth. The lower age limit of 23 days was selected to permit unequivocal identification of glomeruli and assessment of glomerular diameter through the presence of Bowman's space. In dogs of this strain, subcapsular nephrons first appear to be uniformly perfused with ultrafiltrate toward the end of the 3rd wk, and neogenesis of nephrons is completed before this time. The study was extended to 74 days by which time glomerular function expressed per unit of renal weight had reached the mature level (5).

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A 4 mm slice equidistant from both poles was removed from each kidney by cutting it transversely in an anteroposterior direction. The slice was fixed in Bouin's solution for 8 days, dehydrated in alcohol for 8 hr, embedded in paraffin, sectioned serially at 10 μ, and stained with hematoxylin and eosin. Every 10th section was examined. The distance of 100 μ between sections was selected on the assumption that the largest glomerular diameter would not exceed 100 μ so that a glomerulus would be counted only once. Measurements were made in a Zeiss microscope (Ultraphot II) from an image projected on a 9 $\times$ 12 cm screen with 10 μ equal to 0.317 cm.

In each section from a given kidney, cortical areas of uniform width and depth were evaluated. Care was taken to insure that areas did not overlap. Measurements within these areas were conducted in a systematic manner proceeding from the capsule to the medullary border in a line perpendicular to the renal surface. Glomeruli situated on the outer boundaries of an area were recorded only if more than 50% of the tuft was contained inside the area. Approximately 330 glomeruli were examined in each kidney; this number suffices for statistical purposes (4). The volume which contained these glomeruli was calculated from cortical depth, width, number of areas, and thickness of the sections.

Assessment of the total number of glomeruli and of individual glomerular volume was based on the method of Elias and Hennig (4). Diameters of both the glomerular tuft and Bowman's capsule were measured separately with a transparent scale superimposed on the projected image. The largest and the smallest diameters to the nearest 5 μ were recorded for each structure. The average of the two diameters for each structure was termed "glomerular tuft diameter" and "capsular diameter"; simultaneously, the relative cortical depth of each glomerulus was registered. Thus, each glomerulus was characterized by its distance from the capsular surface, its tuft and capsular diameter, and by the age of the kidney. A total of 1652 glomeruli were thus processed. In order to estimate the accuracy of the measurements, 351 glomeruli (approximately 70 per kidney) were recounted at a later date by a different observer. The mean difference between the first and the second measurement was 2.05 μ or 3.0%.

In four additional animals of the same strain, aged 30, 40, 60, and 74 days, the volume of cortex and medulla was determined in both kidneys. Each kidney was dissected free of capsular and hilar fat, weighed on a Mettler H 10 balance, and then bisected in a plane extending through both poles and parallel to the anterior and posterior surfaces. The medulla was separated from the cortex with curved scissors, and both cortical and medullary tissue samples were weighed six times at 10-min intervals. This procedure allowed calculation of water loss with time and therefore extrapolation to the weight of cortex and medulla at the time of removal. At 60 min after removal, the volume of the two medullary and two cortical samples from each kidney was determined by displacement of Ringer's solution in a graduated cylinder. A mean density of 1.0375 was calculated, assuming that the water loss as determined by weight was equally reflected in the measurement of volume.

The data were evaluated by computer analysis for which the cortical histogram at each age was divided into eight equal zones between surface and corticomedullary junction; since the cortex grows with age, these zones increased in absolute size. The analysis yielded the following information: (*a*) the total number of glomeruli per kidney; (*b*) the numerical distribution of glomeruli within the cortex; (*c*) the mean volume of a glomerulus[1] and of a renal corpuscle at each age and in each cortical zone; and (*d*) the total glomerular volume in relation to nonglomerular cortical tissue.

RESULTS

Number and intracortical distribution of glomeruli. The total number of glomeruli for each kidney did not increase with age between 23 and 74 days after birth (Fig. 1). Although the slight decrease from 600 $\times$ 10^3 to 569 $\times$ 10^3 during this period is not statistically significant, an age-related decrease in the total number of glomeruli is a well-known phenomenon and has been observed during late intra-uterine and early postnatal maturation (4, 6, 7). The mean total glomerular count during the postnatal period in the dog strain of the present study was 589 $\times$ 10^3 per kidney. The number of glomeruli per unit volume (mm^3) of cortical tissue decreased 2.7-fold (Fig. 1) almost exclusively because of an increase in cortical volume with age.

The numerical distribution of glomeruli at eight successive levels of the cortex is shown in Fig. 2 *b*. The fraction of the total number of glomeruli within each zone did not vary systematically with age. This suggests that growth of cortical tissue occurred at the same rate in all zones since new glomeruli were not formed after 23 days of age. The fraction and hence the number of glomeruli was highest in level II (24.5%) and decreased within the cortex independently of age to 4.5% in level VIII. The outermost, subcapsular layer (I) of the cortex contained the smallest fraction of the total number of glomeruli; this fraction was nearly 7 times smaller than that in the level directly beneath (II).

Renal weight in this study increased linearly with age (5). However, the relation of cortical and medullary weight to total renal weight was not constant during postnatal maturation. During early maturation, the cortex contributed 61% of the total renal weight and the medulla 39%. By the time the mature relationship (8) was reached at 74 days, the cortical mass constituted 71% and the medullary fraction only 29%. Thus, during the later period of postnatal maturation, the growth rate is higher for cortical than for medullary structures.

Glomerular volume during postnatal maturation. The relation between volume of glomerular tuft and glomerular corpuscle was constant with age at all cortical levels. The glomerular tuft occupied 71.8% $\pm$4.57 (mean $\pm$SEM) of the corpuscular volume.

Although total glomerular volume increased by 33% between 23 and 74 days of age from 0.09 to 0.12 $\times$ 10^3

[1] "Glomerulus" is used as being synonymous with the glomerular tuft, whereas "renal corpuscle" refers to the volume of Bowman's space with its contained glomerular tuft.

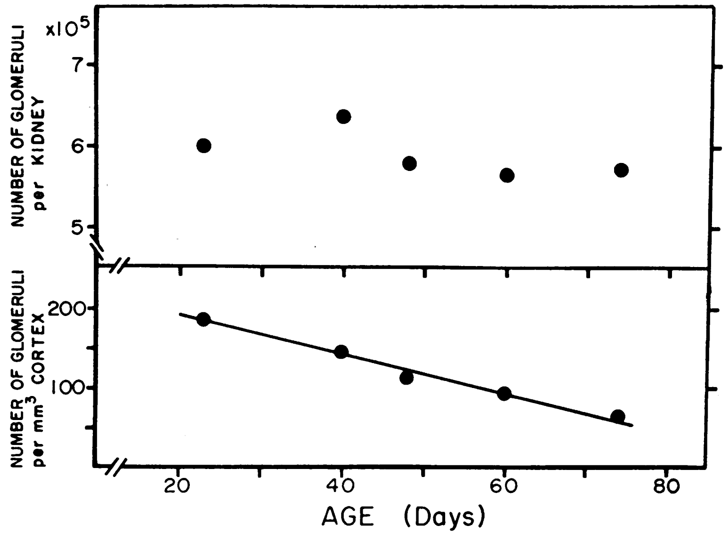

FIGURE 1 Total number of glomeruli per kidney and number of glomeruli per unit of cortical volume during postnatal development in five dogs of a purebred beagle strain.

mm³, nonglomerular cortical volume as estimated by the difference between total cortical and total glomerular volume, rose by 235% from 3.1 to 7.3 × 10³ mm³ during the same period. Consequently, the portion of total cortical volume occupied by glomeruli decreased from 3.8 to 2.3%. On the assumption that nonglomerular cortical tissue consists primarily of tubular rather than interstitial and vascular tissue, these data suggests that tubular growth by far exceeded glomerular growth.

The intracortical distribution of glomerular volume was qualitatively similar in each kidney, i.e., at different phases of postnatal maturation (Fig. 2 *a*). At any age, mean glomerular tuft volume was about 45% greater in the deepest (VIII), juxtamedullary layer of the cortex than in the superficial layers.[3] Except for a slight increment between cortical levels I and II, mean glomerular volume at any one age remained constant down to the juxtamedullary region where it rose sharply. Level VIII probably contains only juxtamedullary nephrons, whereas level VII may contain both type of nephrons. As in the mature kidney of rats and humans (2, 9), in the dog the largest glomeruli were found in the deep cortical region, whereas glomeruli of a smaller and mainly uniform size prevailed throughout the major part of the cortex (Fig. 2 *b*).

The volume of an individual glomerulus varied not only with its position in the cortex but also with age. Within a given layer, the volume of individual glomeruli increased slightly but not consistently with age. In the subcapsular level, this volume rose from 1.38 to 1.89 × 10³ μ³ between days 23 and 74, whereas in the juxtamedullary level it increased at a similar rate from 2.30 to 3.63 × 10³ μ³ during the same time span.

[3] Although the mean individual glomerular volume was largest in layer VIII, the greatest total glomerular volume was located in layer II which contains nephrons of the superficial type.

DISCUSSION

The number of glomeruli in the mature kidney of the dog has been previously assessed. The wide range, i.e. 407 × 10³ (8), 604 × 10³ (10), and 726 × 10³ (11) glomeruli per kidney may have been primarily related to the use of mongrels. This explanation is supported by the constancy of the total number per kidney (589 × 10³) in the five animals of a homogeneous strain reported in the present study. Another factor contributing to the wide range reported previously may have been different adult ages, for a decline in number of glomeruli during adulthood has been observed (12).

Neogenesis of nephrons does not occur in the beagle after 3 wk of age (Fig. 1). This conclusion is based on the findings that the number of glomeruli at 23 days fell within the range reported for mature dogs, the constancy of this number during the subsequent postnatal period, and on the absence of nephrogenic tissue.

The number of glomeruli per unit of cortical tissue declined (Fig. 1) as a consequence of growth of cortical structures. Of these, i.e. tubules, interstitium, vasculature, and glomeruli, the last constituted such a small fraction (3.8–2.3%) of total cortical volume that their contribution to cortical growth may be neglected. Furthermore, the linear decline in Fig. 1 as well as the constancy of the numerical distribution of glomeruli with age (Fig. 2 *b*) suggest that the growth rate of non-glomerular structures was equal and constant in all cortical regions. Thus, one may estimate on the basis of the increase in cortical volume that tubular volume increased by about 235% between 23 and 74 days of age, whereas total glomerular volume as measured directly increased simultaneously by only 33%.

This finding in the dog of a preponderance of tubular over glomerular growth during early postnatal maturation is in agreement with conclusions from an extensive microdissection study (3) in humans. The change in structural balance between glomerulus and tubule in the dog does not, however, result in functional imbalance between glomerulus and proximal convoluted tubule. In the 5 animals reported in this study as well as in 12 others of the same purebred strain, micropuncture analysis of single nephrons showed that proximal fractional reabsorption of sodium and water was constant from 21 to 77 days after birth (5).

The increase in the number of glomeruli per unit of cortical volume between level VIII and II (Fig. 2 *b*) may result from the pattern of centrifugal maturation (1, 2), which leads to a greater predominance of tubular over glomerular volume in juxtamedullary than in more peripheral nephrons.

The dearth of glomeruli in the outermost layer (level

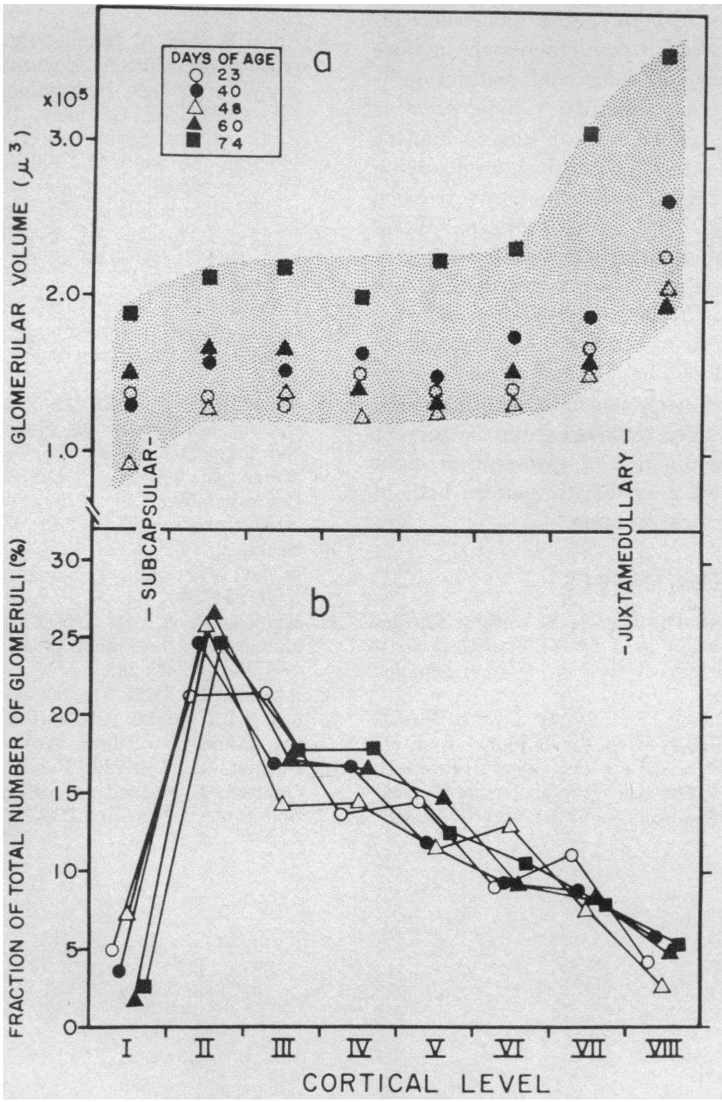

FIGURE 2 Volume of individual glomeruli (*a*) and numerical distribution of glomeruli (*b*) at successive cortical depths during postnatal maturation in the dog. For statistical analysis, the cortical histogram was divided into eight levels equal in volume at any age.

I) may also be related to the centrifugal mode of cortical development. As proximal and distal tubules grow in volume, they might expand preferentially toward the periphery. This process, which would begin at the corticomedullary boundary and progress outward, would necessarily result in an "aglomerular" area beyond the layer of youngest glomeruli, i.e., the subcapsular zone. Thus, the well-known phenomenon that the mature kidney has a layer of renal tissue beneath the capsular surface which contains very few glomeruli is already apparent in the developing kidney (Fig. 2 *b*).

The increase in glomerular function during the 1st yr in humans (13) resembles that of dogs during the postnatal period covered in this and the concurrent functional study (5). That is, in both species glomerular filtration rate *per unit of renal tissue* reaches the mature level. This phase of postnatal functional maturation is accompanied by a change in glomerular volume which is similar for both, namely, an increase of 40% in humans (3) and 33% in dogs. As discussed in the accompanying report (5), however, in neither species is the increase in glomerular volume likely to be a major determinant of the rise in the rate of glomerular filtration.

Microdissection analysis (3) has suggested a marked heterogeneity of nephrons in the human kidney at birth; the same study of nephron maturation at three cortical levels, however, pointed to the attainment of a more regular development at an early postnatal age. Although the first 3 postnatal wk were excluded from the present study in the dog, the examination of glomeruli at eight cortical levels has revealed a systematic pattern both in relation to age and to cortical location.

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