Chronic Reduction of Renal Mass: Glomerular Morphometry by Electron Microscopy

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Changes of glomerular volume in rats were measured up to 21 weeks following subtotal nephrectomy, using morphometric methods. A linear increase of glomerular volume was observed between 2 and 21 weeks after subtotal nephrectomy. This progressive increase in glomerular volume may reflect compensatory hemodynamic changes leading to an increased single nephron glomerular filtration rate.

While unilateral nephrectomy in the rat is known to be followed by early compensatory renal hypertrophy [1], subtotal nephrectomy, by way of contrast, is followed, not only by compensatory renal hypertrophy [2], but also by gradual enlargement of the glomeruli ending in glomerulosclerosis [3] and chronic renal failure [3,4]. The present study was undertaken to quantify the early glomerular changes following subtotal nephrectomy which precede the onset of glomerulosclerosis.

MATERIALS AND METHODS

Experimental animals were female rats of the Sprague-Dawley strain of 180 ± 10g initial body weight. These animals were subjected to subtotal (~5/6) nephrectomy [3] in two operations performed a week apart, in the first of which ~2/3 of the left kidney was resected, and in the second of which the whole right kidney was removed. At intervals of two weeks one experimental and one control rat were anesthetized and the remnant kidney tissue (or left kidney in the control) fixed by arterial perfusion with a tannic acid glutaraldehyde fixative, post-fixed with osmic acid and embedded in an epon-araldite mixture [5]. Electron micrographs were made at low magnification (~350 or ~420) of random ultrathin sections of distinct glomeruli. These sections were mounted on Formvar-coated slot grids, and showed complete profiles of the glomerular sections [5].

Contact prints were used to estimate the mean glomerular volume [E(V)] from the enclosed area (A) and the perimeter (B) of convex contours circumscribed to the images of 20–25 distinct glomeruli from the formula

\[ E(V) = \frac{E(A)}{2E(B^{-1})} \]  
(1)

where \(1/2E(B^{-1})\) represents the mean caliper diameter. The standard error is estimated from the approximation [5]

\[ \text{SE } E(V) = \frac{1}{2E(B^{-1})} \sqrt{\frac{\sum_{i=1}^{n} (A_i - \bar{A})^2}{n(n-1)}} \]  
(2)

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Calibrated enlargements (×6.2 to ×9.4) were used for the measurement of the surface density \( S_v \) of the peripheral glomerular basement membrane (GBM) and the length density \( L_v \) of the peripheral glomerular epithelial slit (GES) as described elsewhere.

RESULTS

Figure 1 shows the regression of glomerular volume on time in the subtotally nephrectomized and in the control groups. The most striking consequence of subtotal nephrectomy is a sustained increase in glomerular volume, from an initial control value of \(-10^6 \mu\text{m}^3\) to a final value of \(-5 \times 10^6 \mu\text{m}^3\) at 21 weeks. There was also a modest reduction at 2 weeks of the \( S_v \) of the peripheral GBM (21.8 percent) and of the \( L_v \) of the GES (15.3 percent) in relation to the initial values for the controls. The further analysis of these changes in density is the subject of another study.

DISCUSSION

It should be remarked that the fixation methods employed and necessary processing precluded a quantitative study of the relative amount of kidney tissue removed in our experimental animals. Our "subtotal nephrectomy" was assumed to remove about 5/6 of kidney tissue on the basis of the surgical protocol [3].

The early enlargement of the glomeruli following subtotal nephrectomy (e.g., at 2 weeks) may reflect in part a passive and possibly reversible change, such as may be implied by the failure of some observers to detect enlargement of isolated, unfixed glomeruli after mononephrectomy in the mouse [6]. It seems less likely that the fivefold enlargement occurring over a period of 21 weeks after subtotal nephrectomy falls into this category.

The mechanism leading to this considerable glomerular enlargement is unknown. Three hypotheses suggest themselves. First, it may represent an aspect of renal hypertrophy occurring in response to functional overload [7]. Secondly, it may be

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FIG. 1. Mean glomerular volume following subtotal nephrectomy. The regression of volume on time is of the form \( y = a + bx \), where \( y \) the glomerular volume measured in \( 10^6 \mu\text{m}^3 \), and \( x \) is time measured in weeks. For nephrectomized animals \( y = 1.869 \pm (0.137) x \), for controls \( y = 0.877 \pm (0.047) x \). The SE of \( a \) is ±0.345 for nephrectomized animals, and ±0.221 for controls; the SE of \( b \) is ±0.027 for nephrectomized animals, and ±0.017 for controls.
part of a renal hypertrophy, occurring as a result of a specific humoral response to the loss of kidney tissue [8]. Finally, it may reflect a glomerular response to hemodynamic changes [9], or to humoral changes inducing them: it is known that there is a prompt compensatory increase in the single nephron glomerular filtration rate (SNGFR) following subtotal nephrectomy in the rat [10].

The first two hypothetical mechanisms would seem to depend on a long-lasting renal hypertrophy following subtotal nephrectomy. While it has been reported that restoration of renal mass is virtually complete 5 days after unilateral nephrectomy [11], the only precise quantitative study of the mass of residual kidney tissue after a subtotal nephrectomy (of an estimated 72 percent of renal mass) gives data only for rats killed 4 weeks after the procedure [2]. At this time there is, proportionally, 75.2 percent restoration of the initial renal wet-weight [2], i.e., an increase to 2.69 times the residual value. The regression equations illustrated in Fig. 1 show that at 4 weeks the glomeruli of our subtotally nephrectomized rats had reached 2.75 times the zero-time control volume by 4 weeks. Thus glomerular and renal hypertrophy 4 weeks after a subtotal nephrectomy would appear to be comparable. It remains to be established whether the renal hypertrophy is sustained, like the glomerular hypertrophy, over much longer periods. It has been clearly established that the compensatory increase in the SNGFR following subtotal nephrectomy in the rat is maintained throughout the period of glomerular enlargement: it lasts at least 30 weeks [10]. Accordingly it is possible to sustain the third hypothesis, that compensatory hemodynamic changes serve as continuing stimulus to glomerular hypertrophy in this system, though the influence of a non-specific or specific stimulus to general kidney growth has not been excluded.

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