Hyperplasia precedes increased glomerular filtration rate in rat remnant kidney

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Hyperplasia precedes increased glomerular filtration rate in rat remnant kidney. Using 3H-thymidine (3H-T), we examined DNA synthesis in rats subjected to either uninephrectomy (UN!), five-sixths nephrectomy (R) or sham (S) surgery. Twenty-four, 48, or 72 hours later, animals were infused with 14C-inulin, PAH and 3H-T and clearances obtained. Prior to sacrifice, India ink was injected for glomerular counting. By 24 hours, glomerular filtration rate per nephron was significantly increased in UN!. However, in R, glomerular filtration rate per nephron was significantly lower than S until 72 hours. Total µg DNA per nephron was unchanged in UN! but significantly increased in R compared to S at all times. 3H-T incorporation into DNA was twice as great in UN! as in S and was over five-fold greater at 24 hours in R than in S; this marked increase persisted in R at 48 and 72 hours. Autoradiographs confirmed that DNA was synthesized predominantly by renal tubular cells and not infiltrating cells. These results indicate that hyperplasia in compensatory renal growth is related to the quantity of tissue removed and that, in the remnant kidney, DNA synthesis precedes the compensatory increase in glomerular filtration rate per nephron.

Acute reduction in nephron mass results in remarkable adaptive structural and functional changes of the remaining tissue. These changes, though initially compensatory in nature, may with time be deleterious and contribute to the progression of chronic renal failure. Although certain functional adaptations are known to occur with decreased renal mass [1–5] and are proportional to the amount of tissue removed [2], it is the increased glomerular hypertension at the nephron level which has received the most recent attention and which is felt to be most injurious [6–8], with resultant compensatory growth and ultimate sclerosis and fibrosis.

Compensatory growth has been most extensively studied in the uninephrectomized animal and, in the adult rat, consists primarily of hypertrophy, with hyperplasia contributing to only about 20 to 30% the increase in renal mass [9–12]. The proportions of hypertrophy and hyperplasia after uninephrectomy are, however, age dependent, with hyperplasia the predominant response until age 14 days whereas hypertrophy predominates after 39 days [13]. Nevertheless, with a sufficiently large stimulus, that is, removal of more tissue [14–16] or administration of folic acid [17], the adult kidney is capable of evoking a greater hyperplastic response.

Five-sixths nephrectomy in the rat results in a remnant kidney which undergoes compensatory changes and eventual renal failure. For this reason it is frequently studied as a model of chronic renal failure. However, little is known regarding the specific characteristics of growth response in this particular model, and it is often assumed that it follows a pattern similar to the uninephrectomy model. The purposes of our studies were to see if hyperplasia in the remnant was a more prominent factor of compensatory renal growth than in the uninephrectomized animal, and to determine its relationship to changes in glomerular filtration rate.

Methods

Surgical Preparation

Ninety-six male, Sprague-Dawley rats (Sasco, Inc., Omaha, Nebraska, USA) weighing 145 to 200 grams were anesthetized with Nembutal (50 µg/g body wt) and divided into three surgical groups. The remnant group consisted of 48 animals subjected to five-sixths nephrectomy as described by Hayslett's group [2, 18], except that all renal tissue was removed in one rather than in two operations. In this group, the right kidney was surgically removed, then broad ligatures to control bleeding were placed around the upper and lower poles of the left kidney and tissue beyond the ligatures excised. The sites of excision were carefully observed to ensure that satisfactory hemostasis was established by the ligatures. Subsequently, at the time of sacrifice, the remnant kidney bed was examined for accumulation of blood from bleeding after surgery. Only animals with insignificant evidence of blood loss were used for further studies. Twenty-four rats underwent total right uninephrectomy and comprised the UN! group, and 24 other rats underwent the same preparatory procedures and served as sham controls (S). Wet weight of the excised renal tissue was measured immediately after surgery, and the animals allowed to recover. Animals then had free access to water, but all were fasted for 24 hours because preliminary experience with R animals showed inconsistent and very poor dietary intake during this time. After fasting, animals were fed 15 grams/day of a commercial diet containing 20% protein (Teklad Test Diets, Madison, Wisconsin, USA); this amount of food was entirely consumed by each animal during the subsequent 24 hours.
Glomerular number and renal function tests

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Hours after surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 ( N = 8 )</td>
</tr>
<tr>
<td>Glomerular number</td>
<td>S-r</td>
<td>29.559 ± 1196</td>
</tr>
<tr>
<td></td>
<td>S-l</td>
<td>29.766 ± 1131</td>
</tr>
<tr>
<td></td>
<td>UNI</td>
<td>30.544 ± 1185</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>11.584 ± 1185</td>
</tr>
<tr>
<td>PAH clearance ( \text{ml/min} )</td>
<td>S</td>
<td>8.79 ± 0.86</td>
</tr>
<tr>
<td></td>
<td>UNI</td>
<td>5.59 ± 0.44</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>1.69 ± 0.21</td>
</tr>
<tr>
<td>Inulin clearance ( \text{ml/min} )</td>
<td>S</td>
<td>2.95 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>UNI</td>
<td>2.00 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>0.45 ± 0.04</td>
</tr>
<tr>
<td>Filtration fraction</td>
<td>S</td>
<td>0.36 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>UNI</td>
<td>0.37 ± 0.02(^{a})</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>0.29 ± 0.02(^{a})</td>
</tr>
<tr>
<td>GFR/nephron ( \text{ml/min/nephron} )</td>
<td>S</td>
<td>50.5 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>UNI</td>
<td>66.5 ± 5.5(^{c})</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>36.7 ± 3.7(^{b})</td>
</tr>
</tbody>
</table>

Abbreviations are: S, sham; r, right; l, left; UNI, uninephrectomy and R, remnant.
Values are the mean ± SEM.

\(^{a}\) NS vs. S of same group;
\(^{b}\) \( P < 0.05 \) vs. S of same group;
\(^{c}\) \( P < 0.02 \) vs. S of same group;
\(^{d}\) \( P < 0.01 \) vs. S of same group;
\(^{e}\) \( P < 0.001 \) vs. S of same group;
\(^{f}\) \( N = 8 \) in R; \( N = 7 \) in S and UNI.

**Clearance and DNA studies**

Eight animals in each group were then studied 24, 48 or 72 hours after surgery. Animals were anesthetized with Inactin (100 \( \mu \)g/g body wt) and placed on a heated table. Rectal temperature was measured with a thermistor probe and maintained between 37.5 and 39°C. The trachea was cannulated with polyvinyl tubing through a tracheostomy, urethra tied, and polyethylene catheters placed in femoral artery and bladder (PE-90) and external jugular vein (PE-10). Clearances of \(^{14}\)C-carboxyinulin (\(^{14}\)C-I; New England Nuclear Corp., Boston, Massachusetts, USA) and \(^{3}H\)-thymidine (\(^{3}H\)-T), specific activity 2.0 Ci/mol (New England Nuclear Corp., Boston, Massachusetts, USA) were determined to measure glomerular filtration rate (GFR) and effective renal plasma flow, respectively. \(^{3}H\)-methyl thymidine (\(^{3}H\)-T), specific activity 2.0 Ci/mmol (New England Nuclear), was used to measure DNA synthesis and clearance of \(^{3}H\)-T. S and UNI animals received a 1 ml priming dose containing 2 \( \mu \)Ci \(^{14}\)C-I, 16 \( \mu \)Ci \(^{3}H\)-T and 3 mg PAH in 0.9% saline, followed by continuous infusion into the jugular vein (pump model 975, Harvard Apparatus Co., Millis, Massachusetts, USA) of 1 \( \mu \)Ci/hr \(^{14}\)C-I, 7 \( \mu \)Ci/hr \(^{3}H\)-T and 3 mg/hr PAH at a rate of 1.1 ml/hr. R animals received a 1 ml priming dose of 1.5 \( \mu \)Ci \(^{14}\)C-I, 12 \( \mu \)Ci \(^{3}H\)-T and 3 mg PAH followed by 0.75 \( \mu \)Ci/hr \(^{14}\)C-I, 5 \( \mu \)Ci/hr \(^{3}H\)-T and 3 mg/hr PAH at 1.1 ml/hr. The lower doses of \(^{14}\)C-I and \(^{3}H\)-T were used in R because of lower renal clearances in this group, so that blood levels similar to the ones present in UNI and S were obtained.

After one hour of equilibration, two consecutive, 30-minute urine collections were obtained under mineral oil in preweighed plastic tubes for clearance measurements. Blood was drawn from the femoral artery catheter midway through each collection period. Average values of the two periods were used for clearance measurements. At the end of the infusion, 1 ml India ink was injected for glomerular counting [2] and animals sacrificed. Removed kidneys were halved and weighed in preweighed plastic cups, with one-half kidney digested overnight at 50°C in 2 ml of 25% HCl for glomerular counting. The other half was frozen in liquid N\(_{2}\), pulverized and the powder weighed in a tared vial containing 5% TCA. After washing twice with cold TCA, DNA was extracted by heating the precipitate in 5% TCA for 30 minutes at 90°C. After cooling, some of the supernatant was assayed for DNA with diphenylamine [19, 20]. Aliquots of tissue, blood and urine samples were added to 10 ml of a scintillation mixture (Instagel or Optifluor, Packard Instrument Co., Downers Grove, Illinois, USA) and DPM’s (disintegrations per min) of \(^{3}H\)-T and \(^{14}\)C-I were counted for 20 min/s sample in a liquid scintillation counter (Packard Tri-Carb).

PAH concentrations in urine and plasma were determined according to previously described methods [21] using a Technicon Autoanalyzer (Technicon Corp., Tarrytown, New York, USA).

The initial weight of excised kidney tissue was known for UNI and R animals. Estimations based upon the assumption that right and left kidney weights in the same animal were approximately equal, and that kidney weights between different animals of similar body weight were approximately equal, were used as previously described [2] to determine the change in remaining wet kidney weight from the time of surgery to sacrifice.
Table 2. Kidney and body weights

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial kidney wt</th>
<th>Final kidney wt</th>
<th>% Change</th>
<th>Initial body wt</th>
<th>Final body wt</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hr (N = 8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>0.83 ± 0.02</td>
<td>0.74 ± 0.02</td>
<td>-11.0 ± 1.9&lt;sup&gt;f&lt;/sup&gt;</td>
<td>176 ± 6</td>
<td>152 ± 5</td>
<td>-13.5 ± 1.0&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>UNI</td>
<td>0.82 ± 0.04</td>
<td>0.81 ± 0.02</td>
<td>-0.8 ± 4.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>173 ± 5</td>
<td>152 ± 5</td>
<td>-12.3 ± 0.6&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>R</td>
<td>0.32 ± 0.03</td>
<td>0.45 ± 0.02</td>
<td>44.1 ± 11.2&lt;sup&gt;f&lt;/sup&gt;</td>
<td>180 ± 3</td>
<td>160 ± 3</td>
<td>-10.9 ± 1.0&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>48 hr (N = 7)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>S</td>
<td>0.77 ± 0.02</td>
<td>0.78 ± 0.05</td>
<td>2.2 ± 6.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>166 ± 6</td>
<td>153 ± 5</td>
<td>-7.8 ± 1.1&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>UNI</td>
<td>0.72 ± 0.03</td>
<td>0.78 ± 0.03</td>
<td>9.5 ± 3.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>159 ± 5</td>
<td>142 ± 4</td>
<td>-10.5 ± 1.2&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>R</td>
<td>0.33 ± 0.02</td>
<td>0.47 ± 0.01</td>
<td>45.0 ± 9.7&lt;sup&gt;f&lt;/sup&gt;</td>
<td>176 ± 5</td>
<td>151 ± 4</td>
<td>-13.8 ± 1.5&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>72 hr (N = 8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>0.78 ± 0.04</td>
<td>0.79 ± 0.03</td>
<td>2.6 ± 6.7&lt;sup&gt;e&lt;/sup&gt;</td>
<td>168 ± 7</td>
<td>154 ± 6</td>
<td>-7.6 ± 1.5&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>UNI</td>
<td>0.78 ± 0.05</td>
<td>0.88 ± 0.04</td>
<td>14.5 ± 6.0&lt;sup&gt;e&lt;/sup&gt;</td>
<td>162 ± 5</td>
<td>152 ± 4</td>
<td>-5.8 ± 1.3&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>R</td>
<td>0.33 ± 0.02</td>
<td>0.55 ± 0.03</td>
<td>67.0 ± 9.0&lt;sup&gt;e&lt;/sup&gt;</td>
<td>172 ± 6</td>
<td>149 ± 4</td>
<td>-13.1 ± 1.1&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Abbreviations are: S, sham (left kidney); UNI, uninephrectomy and R, remnant.

Values are the mean ± SEM.

<sup>a</sup> In the S groups, estimate of initial kidney weight was obtained from the average of the weights of the right kidneys removed from the UNI and R littermates of each S animal.

<sup>b</sup> Final kidney weight in the S group is the weight of the left kidney as measured at sacrifice.

<sup>c</sup> NS initial vs. final wt.

<sup>d</sup> P < 0.05 initial vs. final wt.

<sup>e</sup> P < 0.02 initial vs. final wt.

<sup>f</sup> P < 0.01 initial vs. final wt.

<sup>g</sup> P < 0.001 initial vs. final wt.

In separate studies to evaluate the location of ³H-T uptake within the kidney, additional animals were treated in an identical manner as above except that they received no ¹⁴C-I or PAH and received a higher dose of ³H-T. S and UNI animals received a 1 ml intravenous priming dose containing approximately 0.5 μCi/g body wt of ³H-T (20 Ci/mmol, New England Nuclear) followed by continuous infusion of ³H-T, 0.25 μCi/g body wt/hr at 1.1 ml/hr for two hours, for a total dose of approximately 1 μCi/g body wt. Prime and sustain doses for R animals were reduced by one-third. This resulted in blood levels comparable to those in S and UNI.

When the infusion was completed, animals were sacrificed and kidneys removed. Following fixation in formalin, kidneys were rinsed with water to remove unbound ³H-T, dehydrated with alcohol, embedded in paraffin, cut into sections 5 μ thick, then the paraffin removed with xylene. Autoradiographs were prepared using Kodak N TB-2 liquid emulsion diluted 11 with water. Slides were exposed in light tight sealed boxes in the dark at 4°C for three weeks.

At the end of three weeks, slides were dipped in full strength Kodak D-19 developer for three minutes, rinsed in water, and fixed for three minutes in Kodak Rapid-Fix. Slides were then stained with hematoxylin, and some were counter stained with eosin.

**Statistical analysis**

Data were compared by two-way analysis of variance to detect significant differences in means among the overall groups. Pairs of means were compared using the Student t-test and the Mann-Whitney-Wilcoxon rank sum test. The levels of significance obtained with the two different means tests were almost identical. The results reported are those obtained with the t-test. A P value of <0.05 is considered significant.
Table 3. $^3$H-T + DNA measurements

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Hours after surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 ($N = 8$)</td>
</tr>
<tr>
<td>Blood DPM $^3$H-T</td>
<td>S</td>
<td>15,723 ± 880</td>
</tr>
<tr>
<td></td>
<td>UNI</td>
<td>16,303 ± 830</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>14,306 ± 1698</td>
</tr>
<tr>
<td>$\mu$g DNA/nephron</td>
<td>S</td>
<td>0.09 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>UNI</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>DPM $^3$H-T/$\mu$g DNA</td>
<td>S</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>UNI</td>
<td>2.2 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>5.6 ± 1.1</td>
</tr>
</tbody>
</table>

Abbreviations are: DPM, disintegrations per minute; S, sham; UNI, uninephrectomy and R, remnant.
Values are mean ± SEM.

a NS vs. S of same group
b $P < 0.05$ vs. S of same group
c $P < 0.02$ vs. S of same group
d $P < 0.01$ vs. S of same group

Results

Glomerular number and renal function tests are summarized in Table 1. Number of glomeruli were similar in S and UNI groups and reduced two-thirds in R. Based upon the assumption that right and left kidney weights are similar in the same animal, this resulted in removal of 81, 80 and 79% of total number of glomeruli in 24, 48 and 72 hour animals, respectively. No significant differences were present between the inulin or PAH clearances obtained in the first and second clearance periods. Consequently the results for the two periods were averaged for the data presented in Table 1.

Glomerular filtration rate (GFR) per nephron was significantly increased ($P < 0.02$) as early as 24 hours in the UNI
group compared to S, and remained higher at 72 hours (Fig. 1); at 48 hours the difference between S and UNI was not significant due to a transient increase in GFR in the S group. However, in R animals, GFR was significantly lower until 72 hours, by which time it increased to levels similar to S. GFR per nephron was higher at 48 hours in the S group than at 24 or 72 hours ($P < 0.05$).

Considering that UNI animals had 50% the nephron mass of S, both PAH and inulin clearances were increased relative to nephron mass remaining. These increases were proportional and thus filtration fraction was not significantly different from S at any time studied. In R analysis, however, although PAH clearance was proportional to the amount of tissue remaining, inulin clearance was lower. This resulted in filtration fractions that were lower than S at 24 (NS), 48 ($P < 0.05$) and 72 hours ($P < 0.001$).

Table 2 shows changes in left kidney and in body weights after 24, 48 and 72 hours and indicates the statistical significance of the differences between final and initial measurements within each group. Compared to initial kidney weights, neither S nor UNI animals showed a significant increase in final kidney weight, except for the 48 hour UNI group in which a small increase was present. In contrast, each of the R groups exhibited a weight increase of over 40% between initial and final values.

All animals lost significant body weight. For S and UNI, most of this occurred in the first 24 hours ($P < 0.001$), and although less marked by 72 hours was still significant. R animals, however, had persistent weight loss at 72 hours ($P < 0.001$) which was unchanged from that at 24 hours.

Results of $^3$H-T and DNA measurements are shown in Table 3. As early as 24 hours, total $\mu$g DNA per nephron in R was 1.4 times S ($P < 0.02$) and remained 1.5 times S ($P < 0.01$) by 72 hours (Fig. 2). In UNI animals, total $\mu$g DNA per nephron was not significantly different from S at any time period.

When DNA synthesis was examined by measuring $^3$H-T incorporation into DNA (DPM $^3$H-T per $\mu$g DNA), both UNI and R animals had significantly more DNA synthesis at 24 hours ($P < 0.01$) compared to S, which persisted for at least 72 hours (UNI $P < 0.01$, R $P < 0.02$) (Fig. 3). Though R animals had lower renal clearances of $^3$H-T, blood levels of $^3$H-T were not significantly different from UNI or S at any time period. At 48 hours, there was a decrease in $^3$H-T per $\mu$g DNA in all groups compared to 24 hours; nevertheless, UNI and R remained significantly increased compared to S ($P < 0.01$).

Autoradiographs showed that the greatest labelling in remnant kidney occurred in proximal tubules. After 24 hours, labelling was diffuse throughout the cortex, but by 72 hours was more intense near the margin of ablated tissue (Fig. 4). In 25 randomly selected areas close to the cut margins, at 24 hours there were 22 ± 1.0 labelled nuclei per high power field and 20 ± 1.2 at sites away from the margins. At 72 hours these figures

**Fig. 4. Autoradiograph showing increased labelling of proximal tubule cells near margin of tissue ablation, as well as interstitial cells seen in lower right at 72 hours at 25×.**
Fig. 5. Autoradiograph showing increased labelling of cells in loops of Henle away from margin of tissue injury at 72 hours at 25×.

were 25 ± 3.0 and 20 ± 1.7, respectively. Despite the increased intensity near the margins, labelling away from the margins was also increased compared to controls. Also more prominent at 72 hours than at 24 hours was an increase in labelling of interstitial cells especially in areas near the margin. Loops of Henle were heavily labelled (Fig. 5) as were collecting tubules (Fig. 6). Glomeruli did not show any increased labelling compared to control except for occasional parietal epithelial cells lining Bowman’s capsule. Control kidneys averaged one to two labelled cells per high power field throughout the cortex, whereas remnant kidneys had 20 to 40 labelled cells per high powered field. Labelling in uninephrectomy animals was only slightly more prominent than controls.

Discussion

The capacity for compensatory renal growth (hypertrophy and hyperplasia combined) is an age dependent phenomenon, being greatest in younger animals [22–25]. After nephrectomy this growth consists primarily of hyperplasia in younger animals and hypertrophy in older animals [22, 26–27] with adult patterns reached by about six weeks in the rat [13, 22]. The rats used in the present study were approximately 6-1/2 to 8 weeks old, an age by which adult patterns of growth as characterized by uninephrectomy have been reached. The present study demonstrates that early compensatory growth in the rat remnant model differs from the uninephrectomized model in several ways. Most prominent of these is the greater extent of hyperplasia in the remnant animals. After unilateral nephrectomy, an increase in ³H-thymidine incorporation into DNA occurred, comparable to that observed in previous studies [10, 16]. However, no significant increase in DNA per nephron was detected in the unilaterally nephrectomized groups. This difference between ³H-thymidine results and direct DNA measurements in the uninephrectomy group is due to the much greater sensitivity of the radionuclide technique to detect small changes in the rate of cell division, a consequence of the conservation of nucleotides once incorporated into DNA. In contrast, the remnant groups showed increased DNA content per nephron as well as much greater ³H-thymidine incorporation into DNA, reflecting a much more profound stimulation of cell division. Clearly, uninephrectomy does not evoke the maximum hyperplastic response of which the kidney is capable and compensatory hyperplasia is not only a function of age but of the amount of tissue removed as well. Other data from this laboratory show that as late as four and eight weeks after five-sixths nephrectomy, significant hyperplasia still occurs [28].

The remnant model differs from the uninephrectomized not only in the magnitude of DNA synthesis but also in the time course. Earlier studies have shown that DNA synthesis in the latter rat model peaks at approximately 48 hours [9, 11, 12, 16, 29] and a secondary peak often occurs at 72 to 96 hours [11, 30]. Although the data here show an initial increase at 24 hours and
a second increase at 72 hours, this early increase is consistent with previously reported studies, as there can be much variability in this pattern [14, 31]. Since we did not obtain measurements more frequently than 24 hour intervals, an initial peak at 36 or 40 hours could have been missed, consistent with Williams' report that an initial peak at 40 hours could be entirely missed either eight hours earlier or later [30]. Multiple factors may contribute to the variability reported in previous studies, including the age and sex of the rats, time of day operated on, diet [32] and starvation, which can eliminate compensatory hypertrophic changes [33]. In the remnant however, DNA synthesis was markedly increased as early as 24 hours, and rather than having a secondary peak follow a decrease as in the uninephrectomy group, it remained at the elevated level throughout the time studied. Any effect that initial fasting may have had in suppressing DNA synthesis was clearly overridden by the powerful stimulus of five-sixths renal ablation. In order to keep the role of diet constant in all groups, an initial 24-hour fasting period was used, followed by a modest defined food intake equal in all groups. It is possible that a greater food intake might also have been eaten completely and given rise to higher rates of cell division; however, the relative differences between the groups would probably have been preserved as long as intake remained the same in all groups.

In the remnant group, a significant increase in DNA per nephron was present at the earliest time studied; at 24 hours the remnant nephrons contained 30% more DNA than the controls. Thus, a marked increase in cell division must have occurred in the first 24 hours after five-sixths nephrectomy. The time when this process began and its location within the kidney were not examined in the present study. However, others have found that cell division begins as early as 16 hours after two-thirds nephrectomy [16]. Acute tubular necrosis might have occurred in the first 24 hours, particularly close to the cut margins of tissue, and resulted in increased cell division as part of the restoration process. If so, healing must have occurred before 24 hours since tubular necrosis was not evident in the histologic specimens. Also, the autoradiographic studies showed only a very slight increase in labelling in the regions of the cut margins. Thus, the hyperplastic response present in the remnant kidney between 24 and 72 hours after surgery is predominantly due to a generalized tubular cell proliferation rather than to a localized effect of injury. Since the changes reflecting cell division at 24 hours were similar to those found at later time periods, it is unlikely that pathologic changes prior to 24 hours influenced the results obtained at this time.

Previous studies using the remnant kidney model have found that GFR/nephron four weeks or more postoperatively is over twofold greater than in controls [2, 18]. In contrast, the present study shows that GFR/nephron in the remnant does not exceed that in control kidneys during the first three days after surgery. It should be noted that the GFR results in the sham and unilateral nephrectomy groups are high compared to those obtained in some other studies [5]. If GFR was consistently
overestimated in these groups but not in the remnant kidneys, comparisons of GFR/nephron might be misleading and the major conclusion of this paper would be compromised. Thus the reliability of our GFR measurements is an important issue. There are several considerations which suggest that our measurements are not overestimates of true GFR in the sham and uninephrectomy groups. First, our results for GFR/nephron are very close to those obtained by Kaufman et al [2] on whose work our operative protocol was modeled. These investigators found GFR/nephron to be 47.3 nl/min in controls and 75.9 nl/min in uninephrectomized animals; values for the three time periods in the present study are 50.5, 70.0 and 54.4 in the shams and 66.5, 72.5 and 78.6 in the uninephrectomized groups. Second, the PAH clearances are in line with the inulin clearances so that filtration fractions are appropriate in the sham and uninephrectomy groups. Third, renal function was stable during the clearance measurements since the inulin and PAH clearances did not change between the first and second clearance periods. We conclude, therefore, that the measurements in all groups accurately estimate GFR under the experimental conditions of this study. Whereas GFR/nephron did increase early after uninephrectomy as expected, this response was much slower to develop following five-sixths ablation and remained lower than in controls until the third day. Since neither blood pressure nor blood volume were measured in this study, it is possible that hemodynamic factors were responsible for the low GFR/nephron in the remnant animals. Had measurements been made at later times after operation GFR undoubtedly would have been higher in this group and was beginning to increase at 72 hours. The initial fasting state may have contributed to this delayed increase; however, all animals were fasted for the first 24 hours and less increase in the values in the uninephrectomized animals would be expected if fasting were a factor. Refeeding may have contributed to the increase from 24 to 48 hours in the shams but if this were a general stimulus to increasing GFR/nephron, some increase in GFR/nephron in the remnant group might also be expected at 48 hours.

The observation that GFR/nephron is increased in the remnant kidney several weeks after operation has led to the concept that this change plays an essential role in stimulating cellular growth which accompanies renal ablation [34]. This concept has been used to account for the amelioration of development and progression of renal disease associated with restriction of dietary protein, which reduces GFR/nephron [35, 36]. However, other studies have shown dissociation between compensatory growth and function using ureteral diversion [37] and nephrectomy [38] models. Our data confirm this dissociation between structural and functional changes. The results show that, in the remnant kidney, a powerful stimulus to cell growth rapidly develops and leads to prominent cell division in cortical tubules. At least in the days immediately following ablation, this stimulus is independent of an increase in GFR/nephron.

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**References**