THE EFFECT OF UNILATERAL NEPHRECTOMY ON THE TOTAL NUMBER OF OPEN GLOMERULI IN THE RABBIT

BY ROBERT A. MOORE, M.D.,* AND GREGORY F. LUKIANOFF, M.D.

(From the Department of Pathology, School of Medicine, Western Reserve University, Cleveland)

PLATES 10 AND 11

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Karsner, Bunker and Grabfield (1) and others have demonstrated that removal of one-half the kidney substance in the dog results in a transient renal insufficiency with complete recovery in 1 to 2 days. It is apparent that the opposite kidney compensates for the loss. The majority of the published papers summarized by Hinman (2), deal with blood and urine findings and late hypertrophic changes. So far as we are aware, there is no work to explain the immediate compensation occurring after unilateral nephrectomy.

Through the work of Richards and his collaborators (3) the conception of periodic functioning of glomeruli has been placed on a firm foundation. Hayman and Starr (4) have demonstrated that in general, kidney volume, renal blood flow and urine elimination vary in direct proportion to the total number of open glomeruli.

Method

The technique of Hayman and Starr (4) with some modifications was followed. Rabbits were used throughout. As indicated in the protocols ether anesthesia was employed in some, and in others section of the lumbar spine by the technique of Ecker (5).

In control animals the lower abdomen was opened, the intestines wrapped in a warm towel, the aorta isolated and clamped at the bifurcation and just below the renal vessels, a long slender glass cannula introduced into the aorta between the clamps, the upper clamp removed and the cannula inserted so its tip was at the level of the diaphragm. The outside diameter of the cannula was approximately

* Hanna Research Fellow in Pathology.

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one half of the internal diameter of the aorta. With the cannula in position, the
superior mesenteric artery and coeliac axis were clamped and 0.75 cc. of 3 per cent
solution of Janus Green B (Coleman and Bell) in 0.9 per cent NaCl solution in-
jected under moderate pressure. The time elapsed during the injection averaged
5 seconds. Within 10 seconds the left renal artery was ligated and the animal
killed by a blow on the head or by ether. In some cases the left renal circulation
was irrigated with saline and filled with 5 per cent ammonium molybdate. In
others the left kidney was removed, cut in thin slices and placed in the molybdate
dissolution. After death of the animal the right kidney was injected by the method
of Nelson (6).

In experimental animals, the right kidney with as long a pedicle as possible was
removed through a lumbar incision. It was then injected supravitally with Janus
Green for a determination of the total number of glomeruli. After a variable
period, the left kidney was stained intravitally by the procedure outlined above
for control animals.

In the animals on which a sham operation was performed, the kidney region
was exposed by lumbar incision, the kidney freed from its bed, the pedicle ex-
posed, the whole replaced and in some cases a suspension suture placed in the
capsule. After variable periods these animals were subjected to the same pro-
cedure of intravital staining as the control and experimental group.

The whole procedure may be summed up as follows: One kidney is injected
supravitally to determine the total number of glomeruli in one kidney of this ani-
mal; the opposite kidney is injected intravitally for a determination of the number
of glomeruli which are in active circulation during a period of 5 seconds under
certain experimental conditions: and division of the latter by the former gives
the percentage of open glomeruli.

The counts were made after the general technique of Vimtrup (7). The cortex
and medulla are separated and the weight of the entire cortex secured. From
the cortex, 6 to 10 small samples are removed, pooled and accurately weighed.
The total weight of these samples varied from one-tenth to one-sixth of the entire
cortex. From the number of glomeruli in these samples of known weight, the total
is secured by estimation. In some cases, microscopic sections were also prepared
to determine the completeness of injection in supravitral injections and to check
the percentage injected by the intravital method.

Results

As shown in Table I, in seven adult rabbits, the percentage of open
glomeruli varied from 44 per cent to 78 per cent with an average of
63 per cent. In general those in which ether was used as an anes-
thetic have slightly more than those with fractured spine anesthesia.

After unilateral nephrectomy the percentage was increased to 91
to 99 per cent with an average of 95 per cent (Table II). This
increase appeared in less than 24 hours and continued until at least 10 days after nephrectomy.

### TABLE I

**Normal Controls**

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Supravital Kidney No.</th>
<th>Count</th>
<th>Intravital Kidney No.</th>
<th>Count</th>
<th>Per cent open</th>
<th>Anesthetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45</td>
<td>179,767</td>
<td>46</td>
<td>79,640</td>
<td>44</td>
<td>Section spine</td>
</tr>
<tr>
<td>2</td>
<td>51</td>
<td>185,769</td>
<td>50</td>
<td>101,193</td>
<td>54</td>
<td>Section spine</td>
</tr>
<tr>
<td>3</td>
<td>69</td>
<td>158,400</td>
<td>70</td>
<td>124,410</td>
<td>78</td>
<td>Ether</td>
</tr>
<tr>
<td>4</td>
<td>71</td>
<td>147,734</td>
<td>72</td>
<td>110,670</td>
<td>74</td>
<td>Section spine</td>
</tr>
<tr>
<td>5</td>
<td>76</td>
<td>152,238</td>
<td>75</td>
<td>100,702</td>
<td>67</td>
<td>Section spine</td>
</tr>
<tr>
<td>6</td>
<td>78</td>
<td>138,406</td>
<td>77</td>
<td>92,386</td>
<td>67</td>
<td>Section spine</td>
</tr>
<tr>
<td>7</td>
<td>80</td>
<td>149,134</td>
<td>79</td>
<td>98,210</td>
<td>66</td>
<td>Section spine</td>
</tr>
</tbody>
</table>

Average: ........................................... 63.5

### TABLE II

**Experimental**

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Supravital Kidney No.</th>
<th>Count</th>
<th>Interval in days after nephrectomy</th>
<th>Intravital Kidney No.</th>
<th>Count</th>
<th>Per cent open</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>93</td>
<td>102,300</td>
<td>1</td>
<td>98</td>
<td>99,700</td>
<td>97</td>
</tr>
<tr>
<td>9</td>
<td>94</td>
<td>132,750</td>
<td>2</td>
<td>99</td>
<td>130,111</td>
<td>98</td>
</tr>
<tr>
<td>10</td>
<td>95</td>
<td>170,930</td>
<td>3</td>
<td>100</td>
<td>165,453</td>
<td>92</td>
</tr>
<tr>
<td>11</td>
<td>96</td>
<td>135,670</td>
<td>5</td>
<td>101</td>
<td>122,500</td>
<td>90</td>
</tr>
<tr>
<td>12</td>
<td>97</td>
<td>181,000</td>
<td>10</td>
<td>102</td>
<td>178,420</td>
<td>98</td>
</tr>
<tr>
<td>13*</td>
<td>54</td>
<td>87,350</td>
<td>1</td>
<td>56</td>
<td>80,222</td>
<td>92</td>
</tr>
<tr>
<td>14*</td>
<td>55</td>
<td>111,508</td>
<td>2</td>
<td>58</td>
<td>101,538</td>
<td>91</td>
</tr>
<tr>
<td>15</td>
<td>61</td>
<td>172,331</td>
<td>3</td>
<td>63</td>
<td>169,210</td>
<td>98</td>
</tr>
<tr>
<td>16*</td>
<td>57</td>
<td>68,620</td>
<td>5</td>
<td>60</td>
<td>67,752</td>
<td>98</td>
</tr>
<tr>
<td>17*</td>
<td>62</td>
<td>96,259</td>
<td>10</td>
<td>64</td>
<td>95,499</td>
<td>99</td>
</tr>
<tr>
<td>18</td>
<td>126</td>
<td>150,364</td>
<td>1</td>
<td>106</td>
<td>156,217</td>
<td>100</td>
</tr>
<tr>
<td>19</td>
<td>127</td>
<td>154,782</td>
<td>2</td>
<td>107</td>
<td>149,167</td>
<td>97</td>
</tr>
</tbody>
</table>

* Young animals, 5 to 7 months of age; remainder over 1 year of age.

The animals subjected to a sham operation (Table III) do not differ markedly from the normal controls. The open glomeruli vary from 51 per cent to 75 per cent with an average of 64 per cent.
DISCUSSION

There are certain points in the technique which need further comment, in order to validate the results.

Janus Green B is known to be a powerful vaso-constrictor and it is possible that it might cause such constriction that the results are not those present in life. Hayman and Starr (4) used India ink and obtained the same figures. Further, their results with salt and with caffeine, as well as the results here described following unilateral nephrectomy, prove that 90 to 100 per cent may be open with the same experimental technique.

**TABLE III**

*Sham Operation*

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Supravital Kidney No.</th>
<th>Count</th>
<th>Interval in days after operation</th>
<th>Intravital Kidney No.</th>
<th>Count</th>
<th>Per cent open</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>118</td>
<td>161,208</td>
<td>1</td>
<td>119</td>
<td>82,344</td>
<td>51</td>
</tr>
<tr>
<td>24</td>
<td>104</td>
<td>130,100</td>
<td>1</td>
<td>103</td>
<td>102,614</td>
<td>68</td>
</tr>
<tr>
<td>25</td>
<td>120</td>
<td>158,316</td>
<td>2</td>
<td>121</td>
<td>93,562</td>
<td>59</td>
</tr>
<tr>
<td>26</td>
<td>122</td>
<td>122,488</td>
<td>5</td>
<td>123</td>
<td>86,920</td>
<td>71</td>
</tr>
<tr>
<td>27</td>
<td>124</td>
<td>135,728</td>
<td>10</td>
<td>125</td>
<td>100,786</td>
<td>75</td>
</tr>
</tbody>
</table>

Average........................................................................................................64

The percentage figures depend on the assumption that the two kidneys of any one animal each contain approximately the same number of glomeruli. In addition to the proof offered by Haymann and Starr we add the results in two rabbits.

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Left</th>
<th>Right</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>145,200</td>
<td>152,328</td>
</tr>
<tr>
<td>31</td>
<td>165,160</td>
<td>160,209</td>
</tr>
</tbody>
</table>

We also have unpublished observations that this is true in man and rats. The variation is rarely over 5 per cent. Further proof is adduced by the agreement between the counting method and the study of sections with determination of the relative number stained.

The accuracy of the weight estimation method for determination of the total number of glomeruli may be open to some doubt. We be-
lieve it to be accurate to within 10 per cent. Vimtrup (7) has offered evidence to support this view. Work soon to be published from this laboratory on the total number of glomeruli in the kidney of man and animals will further support the accuracy of the method.

The distribution of the dye in intravitally stained normal kidneys is peculiar. Never are the stained glomeruli distributed evenly throughout the cortex. As noted by Hayman and Starr, often the peripheral glomeruli are not stained while the more central are all stained. In other cases there are small to large areas corresponding to the distribution of an interlobular or interlobar artery in which very few glomeruli are stained. In selecting the specimens for counting, such areas must be taken into consideration and a proportionate amount of them contributed to the pooled specimen.

Richards and Schmidt (3) have noted that an increase in the number of open glomeruli is associated with an increase in the number of open loops of any one glomerulus. In general, we have found this to be true (compare Fig. 3 and Fig. 4). In the active kidney, more glomerular loops are stained and those that are stained are more definite and larger.

The results themselves are unequivocal and need little discussion. The results on the sham operation animals prove that the operation itself is not the cause of the increase. Experiments are under way to contribute evidence on the cause of the increase.

SUMMARY

1. Under the experimental conditions employed, from 44 to 78 per cent of the glomeruli of the normal rabbit kidney contain circulating blood at any one moment.

2. After unilateral nephrectomy the number of glomeruli in the remaining kidney, which contain circulating blood, is increased to 91 to 99 per cent.

3. Compensation for the removal of one kidney is accomplished during the first 10 days at least, by an increase of the number of open glomeruli in the opposite kidney.
REFERENCES


EXPLANATION OF PLATES

PLATE 10

Fig. 1. A teased preparation of cortex injected by Janus Green B, to show the definition of the glomeruli. Such preparations are used in counting. × 8.5.

Fig. 2. Section of kidney supravitally stained. × 110.

PLATE 11

Fig. 3. Section of normal kidney intravitally stained with Janus Green B. Note the unstained and partially stained glomeruli. Compare with Fig. 4. × 90.

Fig. 4. Section of kidney from animal after unilateral nephrectomy intravitally stained with Janus Green B. Note the absence of unstained glomeruli and the more complete staining of the glomerular loops. Compare with Fig. 3. × 90.
FIG. 3

FIG. 4

(Moore and Lukianoff: Unilateral nephrectomy in the rabbit)