It has been known for over a century that when 1 of the kidneys of a laboratory animal has been surgically removed or is not functioning properly, its partner undergoes a compensatory hypertrophy in which both cell size and cell number increase (20, 21, 33). The mechanism of this compensatory growth is unknown. An obvious possibility is that it is due to a single kidney having to carry out the work normally shared between 2. In support of this view, it has been claimed that when normal animals are fed diets containing large amounts of sodium chloride (12) or urea (2, 19, 27) or, in particular, protein (3, 26, 28), their kidneys hypertrophy, presumably in response to the additional load thrown on them. The renal hypertrophy after such a dietary experiment is, however, much slower than that obtained after unilateral nephrectomy.

One difficulty in such investigations has been the lack of a satisfactory method of measuring the extent of kidney growth. The early investigators used kidney weight as a measure of hypertrophy (1, 5). This is obviously unsatisfactory since it gives no indication of the chemical changes occurring or of the relative contributions of changes in cell number or in cell size. It is also rather insensitive, since it can be so easily affected by such factors as the blood or urine content of the kidney. More recently, the rate of kidney growth has been assessed by measuring the changes in mitotic frequency (11, 36).

The present experiments were performed to place the estimation of kidney hypertrophy on a more quantitative basis. For this purpose, the DNA content of the kidney has been used as a measure of the cell number, since the DNA content/nucleus is constant (34); an indication of the average cell composition has then been obtained by relating the other cellular components to DNA. This approach has been used to compare the hypertrophy following unilateral nephrectomy with the variations in kidney size and composition produced by variations in the diet.

MATERIALS AND METHODS

Experimental animals.—The animals used were male albino rats from the departmental colony weighing 130–320 gm and maintained on Diet 41B (4), except in the special dietary experiments. Either right nephrectomy or sham nephrectomy was performed under ether anesthesia between 9:45 A.M. and 12:45 P.M. Right unilateral nephrectomy was performed through a midline abdominal incision.

Diets.—In the special dietary experiments, the animals were fasted overnight and thereafter fed each day at 10 A.M. All animals were offered a fixed intake of diet.
0.3 N KOH were added, and digestion was carried out in a weight tissue) were extracted with 0.5 volume of cold 0.6 N 21% starch, 64% glucose, and no protein. Both diets also contained 5% fat as margarine and 10% of a vitamin-mineral-roughage mixture (22).

The high-protein diet contained 28.5% casein, 7.5% starch, and 49% glucose. The protein-free diet contained 21% starch, 64% glucose, and no protein. Both diets also included 5% fat as margarine and 10% of a vitamin-mineral-roughage mixture (22).

**Tissue preparations and analytical methods.**—At the end of each experiment, control animals were anesthetized with ether and the right kidney was ligated and excised as in unilateral nephrectomy. Each animal was exsanguinated by severing the inferior vena cava and aorta, and the left kidney was then excised. Unilaterally nephrectomized rats were exsanguinated under ether anesthesia by cutting the inferior vena cava and aorta, and the left kidney was excised. The kidneys were blotted on filter paper moistened with isotonic saline, weighed, frozen solid, and stored at −75°C. Nucleic acids were extracted by a modification of the method of Fleck and Munro (7). The kidneys were homogenized in 49 volumes of ice-cold water in a blender, and 5-ml samples (containing 100 mg of wet weight tissue) were extracted with 0.5 volume of cold 0.6 N HClO₄. After standing for 10 min, the precipitate was separated by centrifugation and washed twice with 0.2 N HClO₄. The precipitate (DNA fraction) was made up to 50 ml and a final concentration of 0.1 N HClO₄. The precipitate (DNA fraction) was dissolved in 5 ml of 0.3 N KOH and made up to 25 ml and a final concentration of 0.1 N KOH. The RNA was estimated by measuring the o.d. at 260 μg, and the DNA by the method of Ceriotti (6). They are expressed in terms of ribonucleic acid phosphorus (RNAP) and deoxyribonucleic acid phosphorus (DNAP). Phospholipids were extracted by the method of Folch et al. (9) and estimated by the method of Griswold et al. (13). Total protein was measured by applying the procedure of Lowry et al. (18) to the aqueous kidney homogenate. To estimate protein nitrogen, 0.5 volume of cold 21% trichloracetic acid was added to 5 ml of the aqueous kidney homogenate. After standing for 10 min, the precipitate was separated by centrifugation and washed twice with 7% trichloracetic acid. The precipitate was dissolved in 4 ml of 0.3 N KOH and made up to 8 ml with glass-distilled water. The nitrogen content of the alkaline fraction was estimated by the micro-Kjeldahl method with metallic Hg used as the catalyst. From this the protein nitrogen was obtained by subtraction of the nitrogen content of the nucleic acids present. Thus, protein nitrogen = total nitrogen — (RNAP + DNAP) × 1.69.

**Microscopy.**—Kidneys removed from the rats were fixed in Bouin's fixative, sectioned (7 μ) through the midtransverse region, and stained with hemalum and eosin. The sections were examined under oil immersion, and the number of tubule mitoses in 600 fields (about 40,000 cells) in the cortex of the kidney were counted.

**Statistical methods.**—The statistical significance of the difference between means was assessed by Student's t test. The significance of the differences between the response to operation and to diets in the dietary experiment was assessed by analysis of variance (32).

**RESULTS**

Chart 1 shows the increases in wet and dry weight of the surviving kidney during the 1st 3 days after right unilateral nephrectomy. The wet and dry weights of the left kidney removed at death, which are initially less than those of the right kidney removed at operation, increase steadily to a value about 30% above normal in the 3-day period.

Table 1 shows the increase in mitotic frequency in the surviving kidney over the same period. This increase was not apparent at 24 hr postoperatively, but was clearly

---

**Table 1**

<table>
<thead>
<tr>
<th>No. days after unilateral nephrectomy</th>
<th>Mitoses/10,000 nuclei (mean ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.99 ± 0.39</td>
</tr>
<tr>
<td>1</td>
<td>1.47 ± 0.12</td>
</tr>
<tr>
<td>2</td>
<td>5.31 ± 1.37</td>
</tr>
<tr>
<td>3</td>
<td>9.35 ± 1.72</td>
</tr>
</tbody>
</table>

* Each value represents the mean of 4 kidneys.
The Effect of Right Unilateral Nephrectomy on the Weight, Nucleic Acid Content, Total Protein Content, and Protein Nitrogen Content of the Left Kidney

<table>
<thead>
<tr>
<th>Time after unilateral nephrectomy (hr)</th>
<th>Rat body wt. (gm)</th>
<th>Kidney</th>
<th>Kidney wt. (mg)</th>
<th>DNA±</th>
<th>RNA±</th>
<th>Protein nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>/Kidney</td>
<td>/Kidney</td>
<td>/Kidney</td>
</tr>
<tr>
<td>Unoperated controls</td>
<td>270 ± 8.4</td>
<td>Right</td>
<td>955 ± 29.5</td>
<td>277 ± 8.1</td>
<td>141 ± 12.6</td>
<td>6.149 ± 0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Left</td>
<td>861 ± 26.0</td>
<td>259 ± 5.8</td>
<td>8385 ± 10.2</td>
<td>21.49 ± 0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ratio:</td>
<td>0.90</td>
<td>0.94</td>
<td>0.93</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>to right</td>
<td>± 0.011</td>
<td>± 0.018</td>
<td>± 0.017*</td>
<td>± 0.004</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>Right</td>
<td>716 ± 14.8</td>
<td>216 ± 10.0</td>
<td>304 ± 8.7</td>
<td>14.1 ± 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Left</td>
<td>797 ± 26.1</td>
<td>194 ± 9.5</td>
<td>5379 ± 14.0</td>
<td>19.18 ± 0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ratio:</td>
<td>1.11</td>
<td>0.90</td>
<td>1.25</td>
<td>1.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>to right</td>
<td>± 0.024</td>
<td>± 0.026</td>
<td>± 0.029/</td>
<td>± 0.037/</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>± 0.018/</td>
<td>± 0.013#</td>
<td>± 0.100/</td>
</tr>
</tbody>
</table>

* Nine animals were in the 1st group and 6 animals in each of the 2 other groups. Values are means for the group ± S.E.

The abbreviations used are: DNAP, deoxyribonucleic acid phosphorus; and RNAP, ribonucleic acid phosphorus.

- Ratio significantly different from unity with a P value of 0.001 or less.
- Ratio significantly different from unity with a P value of 0.01 or less.
- Ratio significantly different from unity with a P value of 0.001 or less.
- Significantly different from the ratio for the unoperated control animals with a P value of 0.01 or less.
- Significantly different from the ratio for the unoperated control animals with a P value of 0.01 or less.

established by 48 hr. In agreement with Goss (11), the incidence of mitoses at this point was about 6 times as great as in unoperated controls. There is, however, no apparent peak of mitotic response 40–48 hr after operation, as reported by Ogawa and Sinclair (23) and by Williams (35), nor is the level of mitoses as great as that found by Goss (11). Both these discrepancies may perhaps be explained on the assumption that the response to unilateral nephrectomy varies with the age of the animals (15).

As a preliminary to an investigation of the chemical changes in the surviving kidney after unilateral nephrectomy, a comparison was made of the differences in composition between the right and left kidneys of intact rats. Table 2 shows that on the average the left kidney was lighter than the right and had a lower content of DNA and RNA. The ratios of RNA/DNA and protein/DNA were identical in the 2 kidneys. The assumption that in the rat kidney the DNA content/cell is constant—for which there is ample evidence (34)—means that the left kidney contains fewer cells than the right, though the average protein and RNA content/cell is the same for both kidneys. The differences in the RNA/DNA ratio between the left and right kidneys of any 1 rat was remarkably small—never more than 2%. This means that in unilateral nephrectomy the RNA/DNA ratio of the excised kidney can be taken as an accurate measure of the corresponding ratio for its surviving partner. There was more variation between the right and left kidneys in the protein/DNA ratio, perhaps because this would be affected by the amount of blood present.

Table 2 also shows the results of experiments in which the right kidney (i.e., the larger of the 2) was removed. At 48 hr after the operation the total DNA content of the remaining kidney was still less than that of the kidney removed at operation. The RNA/DNA ratio, however, had increased by about 40%. At 96 hr the total DNA content of the remaining kidney was slightly above that of the excised kidney, but the RNA/DNA ratio was still at the 48-hr level. In other words, the increase in cell number is small even at 96 hr after the operation, whereas there is a much more dramatic increase in the average RNA content/cell. Clearly, therefore, the increase in the RNA/DNA ratio is a much earlier and more sensitive indication of the hypertrophy than the increase in total DNA content/kidney. Table 2 also shows the increase in protein content/cell in the remaining kidney, measured by both the micro-Kjeldahl method and the biuret method of Lowry et al. (18). Both methods give the same results: an increase in the protein/DNA ratio of about 25%, which is substantially less than the increase in the RNA/DNA ratio. Since the method of Lowry et al. is simpler and quicker, it was used in all subsequent estimations.

Table 3 shows the results of a similar experiment in which shorter time intervals were used. In this case, to avoid difficulties due to the difference in size and cell number between the right and left kidneys in intact animals, half the animals in each group were subjected to right nephrectomy and half to left nephrectomy. The results show that at 12 hr and at 24 hr the ratio of the DNA content of the surviving kidney to that of the excised kidney was not on the average significantly different from the corresponding ratio for unoperated control animals (Table 2). But the increase in the RNA/DNA ratio noted at 48 and 96 hr was already perceptible at 12 hr and was fully developed at 24 hr.

If the results of Tables 2 and 3 are considered together, it can be said that after unilateral nephrectomy the surviving kidney shows a small increase in cell number, which is

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Note: The table and text are from the original document, with adjustments for readability and formatting.
detectable by 96 hr but probably not with any certainty at earlier time intervals. A much more dramatic increase in the average RNA content/cell can be demonstrated as early as 12 hr after the operation; it then reaches a plateau level, which is sustained from 24 to 96 hr. At 48 hr a similar but rather smaller increase in average protein content/cell could be detected. Kurnick (16) and Mandel et al. (20) have also shown that the RNA/DNA ratio increases after unilateral nephrectomy.

To test whether the effects of a high-protein diet resemble those of unilateral nephrectomy, an experiment was set up as follows. Twenty-four rats weighing 170—210 gm were divided randomly into 2 groups; 1 was fed a high-protein diet containing twice as much protein as the normal stock diet, and the other was fed a protein-free diet. After 4 days, half the animals in each group were subjected in a random order to right unilateral nephrectomy, and the remainder to a sham operation. After 4 more days on the same diets, all the animals were killed and their kidneys were analyzed. The feeding of the protein-free diet resulted in an average loss of 12 gm in body weight over the 1st 4 days and a further 10-gm loss in the 2nd 4. The body weights of the rats receiving the high-protein diet remained steady over the period of the experiment. These findings are in agreement with those of previous workers (8).

Table 4 shows the results of diet and of right unilateral nephrectomy on the composition of the left kidneys. In both the nephrectomized and the sham-operated group the kidney weights were significantly greater in animals fed the high-protein diet than in those fed the protein-free diet ($P < 0.001$). There was, however, no significant difference in the total DNA content. Apparently, therefore, the diet had affected cell size but not cell number. In each group the animals on the high-protein diet had significantly larger amounts of RNA ($P < 0.01$), protein ($P < 0.001$), and lipid phosphorus ($P < 0.01$) per kidney and significantly larger RNA/DNA ($P < 0.001$), protein/
 effects produced in the remaining kidney after unilateral nephrectomy. The effects of the operation and of a high-protein diet are of the same magnitude as those of a high-protein and lipid phosphorus/DNA (P < 0.001) ratios. Similarly, whether the animals were on the high-protein or the protein-free diet, unilateral nephrectomy increased the kidney weight (P < 0.001) but not the total content of DNA. The total content of RNA (P < 0.01), protein (P < 0.01), and lipid phosphorus (P < 0.001) per kidney and the RNA/DNA (P < 0.001), protein/DNA (P < 0.05), and lipid phosphorus/DNA (P < 0.001) ratios were also increased after the operation. The effects of unilateral nephrectomy in this experiment are therefore broadly in agreement with those previously obtained, namely, a sharp increase in cell content of RNA, protein, and lipid phosphorus, but little or no change in cell number. But it is also clear that over the 8 days of the experiment the protein level of the diet had a marked effect on cell composition, though again not on cell number. Very roughly, the effects of diet are of the same magnitude as the effects of the operation. Moreover, the 2 effects seem to be independent of each other and approximately additive. Konishi (15), using mitotic counts as an index of kidney growth, and Reid (29) and Francis et al. (10), using kidney weight, have also shown that the effects of unilateral nephrectomy and of high dietary protein are additive.

An obvious explanation for the effect of a high-protein diet on the kidney is that it is due to the increased amount of urea that has to be excreted. If this is so, the addition of a substantial amount of urea to the diet should also lead to kidney hypertrophy. Table 5 shows the results of an experiment in which the diet was supplemented with an amount of urea roughly equivalent to twice the protein it already contained. The animals receiving this diet therefore presumably had to excrete 3 times the normal daily amount of urea. Over a 4-day period on this diet there were significant increases in the RNA/DNA (P < 0.002) and protein/DNA (P < 0.05) ratios. There was no significant difference in the kidney weight or in the total DNA content. Although these changes are similar to the effects produced in the remaining kidney after unilateral nephrectomy, they are only about \( \frac{1}{3} \) as great (Table 5). It seems unlikely, therefore, that the hypertrophy of the remaining kidney that follows unilateral nephrectomy is due simply to the increased amount of urea it has to excrete.

## DISCUSSION

The results described above show that during the 4 days following unilateral nephrectomy the surviving kidney increases in size by nearly 30%. Particularly during the 1st 2 days this increase reflects an increase in the average cell size and content of RNA and protein. The increase in cell number is much slower. It is too small to be easily detected 2 days after the operation, and at 4 days it amounts only to about 10%. The mitotic counts shown in Table 1 indicate that mitotic activity is not significantly increased until the 2nd day after the operation. This can be contrasted with the increase in RNA/cell, which is detectable at 12 hr and is almost fully developed at 24 hr. There is an interesting comparison here with liver regeneration after partial hepatectomy, in which there is also a marked postoperative increase in RNA/cell. This is, however, accompanied by an almost equivalent increase in DNA/cell and is followed within 24–48 hr of the operation by a tremendous increase in mitotic activity (14). This difference between the 2 tissues is consistent with the view that liver regeneration is, from the beginning, due chiefly to an increase in cell number, whereas compensatory hypertrophy in the kidney is, at least in the early stages, due chiefly to an increase in cell size.

From a practical point of view the RNA content/cell (i.e., the RNA/DNA ratio) should provide a sensitive means of detecting and measuring compensatory hypertrophy of the kidney within 24 hr after unilateral nephrectomy. It has the great advantage of being virtually identical in the 2 kidneys of the same animal, and after unilateral nephrectomy it shows a greater increase than any of the other tissue components, but its usefulness in this respect might seem to be limited by the degree to which it is affected also by the protein content of the diet. Indeed, one of the most striking results to emerge from the present investigation is that the variations in kidney com-

### TABLE 5

<table>
<thead>
<tr>
<th>Diet</th>
<th>Rat body wt. (g)</th>
<th>Treatment</th>
<th>Kidney wt. (mg)</th>
<th>DNA*</th>
<th>RNAP</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>41B</td>
<td>157 ± 8.0</td>
<td>Left</td>
<td>579 ± 27</td>
<td>274</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td>41B + 10% urea</td>
<td>157 ± 8.3</td>
<td>Left</td>
<td>627 ± 16</td>
<td>300</td>
<td>108</td>
<td></td>
</tr>
<tr>
<td>41B</td>
<td>146 ± 5.8</td>
<td>Unilateral nephrectomy</td>
<td>612 ± 35.4</td>
<td>299</td>
<td>103</td>
<td></td>
</tr>
</tbody>
</table>

* The 1st 2 groups each contained 6 animals; the nephrectomized group contained 5 animals. Values are means for the group ± S.E. For the purpose of statistical comparison the animals were paired according to body weight. The urea diet consisted of the basal Diet 41B (protein content, 13.7%), containing 10% urea by weight. The feeding of this diet gave a nitrogen intake of 3400 mg over the 4 days of the experiment; the nitrogen intake on the basal diet was 1100 mg over the same period.

* The abbreviations used are: DNAP, deoxyribonucleic acid phosphorus; and RNAP, ribonucleic acid phosphorus.
position produced by varying the protein content of the diet resemble those which follow unilateral nephrectomy.

Inevitably, the close resemblance between the effects of diet and of unilateral nephrectomy gives some support to the view that after unilateral nephrectomy the surviving kidney grows because it has to excrete double its normal load of urea. This seems unlikely, however, since the effects of feeding a diet containing urea on kidney size and composition (nitrogen intake, 3400 mg in 4 days, Table 5) are so much less than the effects of unilateral nephrectomy on the remaining kidney (nitrogen intake, 1100 mg in 4 days, Table 5). Similarly, the effects of variation in protein intake on the kidney cannot be attributed solely to the consequent variation in the amount of urea that has to be excreted.

It could seem more reasonable to assume that the effects of high-protein intake and unilateral nephrectomy, in spite of their apparent similarity, are probably quite distinct. The increase in the RNA and protein/kidney cell after unilateral nephrectomy can be regarded simply as the 1st stage in the incipient compensatory growth. The similar increase produced by increasing the protein intake is more probably an aspect of the well-known relationship between the protein intake and the protein content of both liver and kidney (23).

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Chemical Aspects of Compensatory Renal Hypertrophy

I. W. Halliburton and R. Y. Thomson


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