The Effect of Diet and of Unilateral Nephrectomy on the Composition of the Kidney

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SUMMARY

In rats, unilateral nephrectomy is followed within 36 hours by a fall in the DNA concentration and a rise in the RNA/DNA and protein/DNA ratios in the remaining kidney. The changes in DNA concentration and in RNA/DNA ratio take place regardless of whether the animal is fed or fasted postoperatively; the increase in protein/DNA ratio takes place only if the animal is fed. Changes similar to but not identical with those produced by unilateral nephrectomy can be produced in the kidneys of intact rats by administering ammonium chloride in the diet for 6 days in amounts sufficient to cause acidosis. Equivalent amounts of sodium chloride or ammonium citrate do not produce these effects.

INTRODUCTION

The removal of one kidney of a laboratory animal is followed by a compensatory growth of the other (10, 29). Although the control of this renal growth has been studied for over a century, there is still no clear indication of the mechanism involved. The majority of investigators who have tried to explain compensatory renal hypertrophy have favored the idea that it is a “work hypertrophy,” i.e. a response to a situation in which one kidney has to cope single-handed with the excretory functions normally shared between two. The assumption implicit in this view is that the size of the kidneys is determined, at least in part, by the amount of material they have to excrete. Attempts have been made to demonstrate this by severing one ureter in an experimental animal and either allowing it to drain into the peritoneal cavity (11, 21, 26, 28) or implanting it into the duodenum (5, 6, 9).

In either case, the urine secreted by the corresponding kidney should be reabsorbed into the circulation so that, although both kidneys are intact, only one functions effectively. In theory, such experiments are beyond criticism. In practice, however, there are grave technical difficulties. Urine discharged into the peritoneal cavity is not, in general, completely reabsorbed (28) and in any case, its presence leads to inflammation which appears to have side effects on the kidney (26). It is also possible that the severed ureter may become inflamed or blocked. Such experiments have, therefore, doubtful significance, and it is not surprising that they have not given clear-cut results.

Alternatively, it should be possible to influence kidney function in the intact animal by supplying in the diet an excess of material which must be excreted via the kidneys. According to the hypothesis, this should cause renal hypertrophy, and it has been reported that diets containing large amounts of salts (11, 17) or urea (3, 14, 23) or protein (13–15, 18, 24) in fact do so. The results have not, however, been clear-cut. A fundamental difficulty has been the use of kidney weight or mitotic index as the main indicator of kidney growth. The shortcomings (e.g. smallness and slowness of the changes) of these methods have been discussed in a previous paper (14).

More recently, the growth of the kidneys has been followed by using their DNA content as a measure of cell number and as a means of calculating the average cell mass and the amount of each constituent per cell (13, 14, 16, 17, 20). The advantage of this method lies in the fact that while the two kidneys of a single animal may differ substantially in size, this difference is due solely to difference in cell number. The average amount of each constituent per cell in the two kidneys is virtually identical (14). Therefore, the composition per cell in the kidney removed at unilateral nephrectomy can confidently be taken as representing the composition per cell of the remaining kidney at the time of operation. If, some time after the operation, the animal is killed and the remaining kidney analyzed, comparison with the kidney excised at operation will give a very precise indication of the changes which have taken place between operation and death. By this means, Halliburton and Thomson (13, 14) and Mandel and his associates (19, 20) have shown that one of the earliest and most dramatic of these changes is a substantial increase in RNA content per cell.

The object of the experiments described in the present paper was to clarify the relationship between compensatory renal hypertrophy and the effects of diet. Answers were sought to two specific questions. The first of these was whether starvation after unilateral nephrectomy inhibits compensatory renal hypertrophy. Williams (33) has shown that mitotic activity in the kidney remaining after unilateral nephrectomy is greatly depressed in starved rats and Royce (26) and Reiter (25) both found that if rats were deprived of food and water after unilateral nephrectomy, the surviving kidneys did not show an increase in weight or in DNA. The second question is whether the addition of salts to the diet, with subsequent changes in the volume and composition of the urine, and therefore in the excretory function of the kidney, has any effect on kidney size and composition.

MATERIALS AND METHODS

Experimental Animals. The animals used were male albino rats from the departmental colony weighing 140–215 gm main-
tained on Diet 41 (7) unless otherwise stated. Right unilateral nephrectomy was performed through a midline abdominal incision. All operations were performed between 9.30 a.m. and 12.30 p.m.

Diets. The animals were fed Diet 41 (7) supplemented with various salts or with urea as indicated. Water was supplied ad libitum. The animals were fed each day at 10 a.m.

Tissue Preparations and Analytic Methods. At the end of each experiment the animals were anesthetized with ether, the right kidney ligated and excised as in a unilateral nephrectomy, and the left kidney treated in exactly the same way. Unilaterally nephrectomized animals were anesthetized with ether and the left kidney ligated and excised. The kidneys were blotted on filter paper, moistened with isotonic saline, weighed on a torsion balance, frozen solid, and stored at —75°C. DNA, RNA, and protein were estimated as described by Halliburton and Thomson (14). DNA was expressed in terms of deoxyribonucleic acid phosphorus (DNA-P) and RNA in terms of ribonucleic acid phosphorus (RNA-P). In experiments involving examination of urine, the animals were kept in metabolic cages (31), and urine was collected in 24-hour periods using chloroform as a preservative. Urinary urea was estimated by the hypobromite method using the Doremus Ureometer (32). Urinary ammonia was estimated by a formol titration. Two ml of urine were diluted 1 in 10 with distilled water. Two drops of phenolphthalein were added followed by 0.1 N NaOH from a buret until a stable pink color was obtained. Neutralized formaldehyde solution (1 ml 40% (w/v) formaldehyde neutralized with 0.1 N NaOH) was added and the mixture titrated with 0.1 N NaOH to the same pink color as before. Since in this formol titration one NH₃ group yields one equivalent of hydrogen ion, then 1 ml of 0.1 N NaOH is equivalent to 1.4 mg ammonia.

Statistical Analysis. The statistical significance of the difference between means was assessed by Student's “t” test (30).

RESULTS

The effect of starvation was investigated in thirty-six rats (weighing 140 to 150 gm) which were subjected to right unilateral nephrectomy. They were then divided randomly into two groups. Postoperatively, one of the groups was given food ad libitum, the food intake of each individual animal being measured; the other group was starved. After 12, 24, and 36 hours, six animals from each of the groups were killed and their remaining kidneys analyzed. Six unoperated animals were also sacrificed as a zero time control. Comparison of the composition of the remaining kidney at death with that of the kidney removed at operation indicated the changes which had taken place in the remaining kidney since the operation. The results are shown in percentage form in Chart 1. In the fed animals, unilateral nephrectomy produced no significant changes in the composition of the remaining kidney during the first 12 hours, but at 24 hours there was a 21% increase in RNA/DNA (P < 0.001) and a 12% increase in protein/DNA (P < 0.05) ratios and a 12% fall in the amount of DNA per 100 mg (P < 0.05). At 36 hours these changes were all accentuated. The RNA/DNA ratio was 33% above control level (P < 0.001), protein/DNA ratio was 17% above control (P < 0.001), and the DNA content per 100 mg was 19% below control level (P < 0.01). Assuming that the DNA content per cell is constant in the kidney (14), these results mean that unilateral nephrectomy caused the cells of the remaining kidney to increase in mass and in content of RNA and protein. This is in agreement with results previously reported (13, 14, 19).

In the fasted animals again no significant changes could be seen at 12 hours. At 24 hours there was a 16% increase in RNA/DNA ratio (P < 0.001). This is smaller than the corresponding increase for the fed animals at this time interval but not significantly so. At 36 hours, the RNA/DNA ratio for the fasted animals was 22% above the control level (P < 0.001); again this is smaller than the corresponding increase for the fed animals but again the difference is not significant. A completely different picture is obtained with protein/DNA ratio, which shows no significant increase at all in the fasted animals either at 24 or at 36 hours. The difference in this respect between the fed and fasted animals is significant at 36 hours (P < 0.01). The DNA content per 100 mg in the fasted animals shows a pattern intermediate between RNA/DNA ratio and protein/DNA ratio. It does not show a significant change until 36 hours and at this point is above the corresponding figure for fed animals (P < 0.05). Clearly, fasting modifies the response to unilateral nephrectomy by abolishing the accumulation of protein in the cells of the remaining kidney and perhaps also by diminishing the increase in cell mass which would otherwise take place. It does not, however, abolish the increase in DNA per cell which has previously been shown to be one of the earliest and most dramatic manifestations of compensatory renal hypertrophy (14). This is a little surprising. It has been known for many years that starvation does diminish the protein content of some organs, notably the liver (1, 2, 22), but, generally speaking, the fall in protein per cell is accompanied by an equivalent fall in RNA per cell (4). In the present instance, however, it is important to remember that the animals were starved for only a very short period and that if starvation had been prolonged, additional changes in composition, including an effect on RNA, might have become apparent. It is clear, however, that the early changes of compensatory renal hypertrophy are not inhibited by starvation or even significantly depressed by it. It does look, therefore, as though the early stages proceed without much regard to the nutritional status of the animal.

The effect of salt intake on kidney size and composition was investigated by adding sodium chloride, ammonium chloride, and ammonium citrate to the diet. Sodium chloride was chosen because electrolyte balance is largely a matter of sodium excretion or retention. In addition, sodium ions are the main cations of the urine. Adding sodium chloride to the diet would therefore increase the amount of sodium which the kidneys would have to excrete, and this in turn would increase the water output. Ammonium chloride was used since it is well known to produce acidosis, for in the conversion of the ammonium ion to urea, a hydrogen ion is released. Ammonium citrate was used as a control for the ammonium chloride fed group. This salt would be expected to be completely metabolized in vivo. The ammonium ions are converted to urea with the release of a hydrogen ion, but this would be balanced by the uptake of hydrogen ions associated with metabolism of citrate to CO₂ and H₂O.

The concentrations of these salts in the diets used were dictated by the amounts which the animals would tolerate. Sodium chloride and ammonium citrate were fairly well tolerated. Diets

Effect of Diet or Unilateral Nephrectomy on Kidney
Chart 1. The effect of starvation on the body weight and on the composition of the remaining kidney of male rats (body weight 140-150 gm) which had been subjected to right unilateral nephrectomy. Each point represents the mean for 6 rats; vertical bars represent S.E. The changes in kidney composition are expressed as the percentage difference between the remaining kidney removed at death and the kidney excised at operation. DNA-P and RNA-P, deoxyribonucleic and ribonucleic acid phosphorus.

containing substantial amounts of ammonium chloride were not; 3% by weight was the maximum the animals would accept. Accordingly this was used. The ammonium citrate diet used (7% by weight) gave a nitrogen intake equivalent to that of the ammonium chloride diet. The sodium chloride diet used (3.3% by weight) was equimolar to the 3% ammonium chloride diet. These three diets were fed to groups of six animals for a period of six days, and the size and composition of the left kidneys was...
Effect of Diet or Unilateral Nephrectomy on Kidney

Chart 2. The effect of high-salt diets and of a urea-containing diet on urine volume per day. In each of the first 3 groups, each point represents the mean for 3 animals weighing between 165 and 180 gm. In the fourth group, each point represents the mean for 6 animals weighing between 146 and 152 gm. The animals were fed 12 gm of the stock diet (7) per day for 4 days. Between the periods marked by arrows, the animals were fed diets as indicated. The sodium chloride diet contained 96.7% by weight stock diet and 3.3% sodium chloride. The ammonium chloride diet contained 97% stock diet and 3% ammonium chloride. The ammonium citrate diet contained 90% stock diet and 7% ammonium citrate. The urea-containing diet consisted of 90% stock diet and 10% urea.

Chart 3. The effect of high-salt diets on the daily urinary ammonia output. Each point represents the mean for 3 animals weighing between 165 and 180 gm. The diets used were as described in Chart 1. Between the periods marked by arrows, the animals were fed diets as indicated.

The effect of these diets on kidney composition is shown in Table 1. The sodium chloride diet had no apparent effect on kidney size or composition. Thus the amount of sodium or chloride ions to be excreted has no effect on the size of the kidney, nor indeed has the amount of water to be excreted. This latter conclusion can also be reached in considering the effect of a 10% urea diet. This diet resulted in a 4-fold increase in urea output (Chart 4) and in a roughly proportional increase in urine volume (Chart 2). The effect of the diet on kidney composition has, however, previously been shown to be very slight (14); the only effects were an 11% increase in RNA/DNA ratio ($P < 0.002$) and a 7% increase in protein/DNA ratio ($P < 0.05$) which were only about one-third as great as the effects of unilateral nephrectomy (14). The ammonium chloride diet, on the other hand, did have a marked effect on the kidney, producing a significant increase in kidney weight ($P < 0.001$). This was not due to an increase in cell number, for the total content of DNA per kidney did not alter, but the DNA concentration decreased ($P < 0.05$). There was also an increase in the total content of RNA ($P < 0.001$) and of protein ($P < 0.01$) and in RNA/DNA ($P < 0.02$) and protein/DNA ($P < 0.05$) ratios. The ammonium citrate diet produced only a 12% increase in kidney weight ($P < 0.05$) and an 11% increase in the total content of RNA ($P < 0.05$). Comparison of the animals fed ammonium chloride with those fed ammonium citrate showed significant differences in kidney weight ($P < 0.02$), RNA/kidney ($P < 0.02$), protein/kidney ($P < 0.01$), and protein/DNA ratio ($P < 0.05$). Thus, the kidney hypertrophy produced by the ammonium chloride diet cannot be due to increased electrolyte excretion or to increased urea excretion or to increased water excretion, and must therefore be a result of the acidic effect of the ammonium chloride.
DISCUSSION

In normal unoperated rats subjected to starvation, Kurnick (16) has shown that there is a reduction in total RNA and protein content per kidney during the first 2 or 3 days but no change in the total DNA content per kidney or per cell, indicating that the cells are diminishing in size but not in number. The first report of the effect of starvation on compensatory renal hypertrophy was that of Sacerdotti (27) who stated that it was inhibited. Hall and Hall (12) also found that the increase in kidney weight following unilateral nephrectomy was almost completely suppressed if the animals were fasted. In agreement with this, Williams (33) has shown that the mitotic activity in the kidney remaining after unilateral nephrectomy is greatly depressed in starved rats. More recently, Royce (26) and Reiter (25) both found that if rats were deprived of food and water after unilateral nephrectomy, the surviving kidneys did not show an increase in weight or in DNA synthesis. It may, however, be misleading to say that starvation “inhibits” compensatory renal hypertrophy. It would be more accurate to say that starvation tends to make normal kidneys diminish in size and mitotic activity (16, 33). Unilateral nephrectomy tends to make the remaining kidney grow. When a rat is subjected to unilateral nephrectomy and at the same time deprived of food, the two effects roughly cancel out and the remaining kidney remains the same size as before. However that may be, the important practical conclusion that comes out of the present experiment is that the increase in RNA content per cell, which is the most dramatic early change in compensatory renal hypertrophy (14, 19), is not significantly affected by starvation. To that extent the RNA/DNA ratio is a more reliable indicator of compensatory renal hypertrophy than is mitotic index or increase in kidney weight. It is also of interest that whereas starvation has been shown virtually to inhibit the increase in mitosis in the remaining kidney after unilateral nephrectomy (33), it apparently does not affect the increase in cell number in the remaining liver fragment after partial hepatectomy (8).

Although starvation does not prevent all the chemical changes which accompany compensatory renal hypertrophy, it is clear that increases in kidney cell size and composition can be brought about not only by unilateral nephrectomy but also in response to variations in the diet. The results described above show that the addition of ammonium chloride to the diet of normal intact rats results in a marked increase in mean cell mass and in content of RNA and protein, whereas equivalent amounts of sodium chloride and ammonium citrate do not; nor does urea. These findings can be explained on the basis that acidosis produced by ammonium chloride (or other means) necessitates a greatly increased renal production of ammonium ions from glutamine (Chart 3). Such a large increase in one of the chemical activities of the cells might be expected to evoke a change in their pattern of enzyme synthesis sufficient to account for the observed increase in protein and RNA. Excretion of additional amounts of sodium chloride or urea would not call for any comparable alteration in the chemical activities of the kidney cells. Dietary ammonium citrate presumably is metabolized completely to CO₂, water, and urea, and would therefore not be expected to make any chemical demands on the kidney cells either.

Whatever the explanation of the effects of ammonium chloride it is important to decide how far they are identical with the changes which follow unilateral nephrectomy. There does seem to be one important difference. After unilateral nephrectomy the increase in RNA in the surviving kidney is normally much greater than the increase in protein. This is reflected in a steady increase in the RNA/protein ratio (Chart 5). Dietary ammonium chloride, on the other hand, increases RNA and protein to roughly the same degree, so that the RNA/protein ratio does not alter (Table 1). The two responses, therefore, do not seem to be identical.

The conclusion to be drawn from the present experiments therefore would seem to be that the growth of the remaining kidney after unilateral nephrectomy cannot be explained on the ground that it has to excrete more urea, more salt, more water, or more acid.
TABLE 1
The Effect of High-Salt Diets on the Weight and Composition of the Left Kidney after 6 Days

<table>
<thead>
<tr>
<th>Diet</th>
<th>Left kidney wt. (mg)</th>
<th>DNA-P*</th>
<th>RNA-P</th>
<th>Protein</th>
<th>RNA-P/protein ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>± S.E.</td>
<td>μg/100 mg wet wt.</td>
<td>μg/kidney</td>
<td>μg/mg DNA-P</td>
<td>μg/mg DNA-P</td>
</tr>
<tr>
<td>Diet 41c</td>
<td>663 ± 27.0</td>
<td>32.2 ± 1.4</td>
<td>213 ± 12.9</td>
<td>317 ± 10.3</td>
<td>1.50 ± 0.07</td>
</tr>
<tr>
<td>3.3% sodium chloride</td>
<td>708 ± 24.4</td>
<td>33.4 ± 1.1</td>
<td>237 ± 14.8</td>
<td>341 ± 10.8</td>
<td>1.46 ± 0.05</td>
</tr>
<tr>
<td>3% ammonium chloride</td>
<td>835 ± 21.8*</td>
<td>27.2 ± 1.1*</td>
<td>226 ± 8.2</td>
<td>398 ± 12.1*</td>
<td>1.77 ± 0.05/</td>
</tr>
<tr>
<td>7% ammonium citrate</td>
<td>744 ± 15.6*</td>
<td>30.4 ± 1.2</td>
<td>225 ± 7.3</td>
<td>352 ± 8.6*</td>
<td>1.58 ± 0.07</td>
</tr>
</tbody>
</table>

* Values are means ± S.E. for 6 animals weighing 175-215 gm. They were offered 12 gm of diet per day. The first group therefore received 12 gm of stock diet (7) per day; the second group received 11.60 gm stock diet and 0.40 gm sodium chloride; the third group received 11.64 gm stock diet and 0.36 gm ammonium chloride; and the fourth group received 11.16 gm stock diet and 0.84 gm ammonium citrate.

\* The abbreviations used are: DNA-P, deoxyribonucleic acid phosphorus; and RNA-P, ribonucleic acid phosphorus.

\* See Ref. 7 for details.

\* Significantly different from the value for the animals on the stock diet with a P value of 0.001 or less.

\* Significantly different from the value for the animals on the stock diet with a P value of 0.05 or less.

\* Significantly different from the value for the animals on the stock diet with a P value of 0.02 or less.

\* Significantly different from the value for the animals on the stock diet with a P value of 0.01 or less.

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CHART 5. The effect of unilateral nephrectomy on the ribonucleic acid phosphorus (RNA-P) protein ratio of the remaining kidney, expressed as a percentage of the ratio for the excised kidney. The points at 0, 1, 2, and 4 days are the means for 10 animals, those at 3 and 41 days are the means for 6 animals (body weight 140-200 gm); vertical bars represent S.E.

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