Renal hypertrophy in streptozotocin-diabetic rats

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Summary
1. Kidney weight and content of protein, RNA and DNA were measured in rats with streptozotocin diabetes of varying duration.
2. Diabetic rats had larger kidneys than control rats: after 3 days of diabetes the weight increase was 15% and after 42 days of diabetes it was 90%. The protein content rose in parallel to the weight.
3. RNA content was already increased after 36 h of glycosuria, whereas DNA content was unchanged for the first 3 days of diabetes, and increased thereafter. The protein/DNA ratio increased rapidly during the first 3 days but remained constant thereafter.
4. Insulin treatment decreased the renal weight gain by about 67% during the first 8 days of diabetes, but did not prevent the increase in DNA. When insulin was started after 25 days of diabetes there was only a slight regression of kidney growth.

Key words: deoxyribonucleic acid, diabetes, hyperplasia, hypertrophy, kidney, ribonucleic acid.

Introduction
Patients with diabetes mellitus of recent origin have a higher glomerular filtration rate than non-diabetic subjects (Mogensen, 1971) and recently Mogensen & Andersen (1973) have demonstrated that the kidney is larger in diabetes mellitus. Furthermore, both renal function and kidney size return to normal after strict insulin treatment (Mogensen & Andersen, 1975).

Increased kidney size has also been reported in the diabetic rat, but this phenomenon has not been systematically investigated. The present study aims to describe kidney growth in diabetic rats in terms of time-course, hypertrophy and hyperplasia.

Methods and materials
Female Wistar rats were made diabetic by the intravenous injection of streptozotocin (40 mg/kg). Only rats who had glycosuria within 24 h after the injection, and who had a blood glucose of 19.5 mmol/l or more at the time of killing, were included in the experiment. None of the animals had ketonuria at the termination of the experiment.

All rats used in a single experiment were of the same size, and did not differ by more than 10 g in body weight from each other at the start of the experiment. Unless stated otherwise all rats were between 4 and 5 months old.

Four different studies were performed. First, groups of animals were investigated 3, 8, 12 and 42 days after the induction of diabetes and compared with a control group of normal animals. As these experiments showed renal hypertrophy to be present even after 3 days, the other groups of rats were studied after shorter intervals. To circumvent any possible acute toxic effects of streptozotocin animals were treated with protamine-zinc insulin (2 units every 12 h) and maintained glycosuria-free 3 days after streptozotocin. Thereafter the rat’s urine was tested every 2 h for glucose. Groups of rats were studied 12, 24, 36, 48 and 60 h after the appearance of glycosuria.

In a third set of experiments the effect of insulin treatment on kidney hypertrophy was studied. One
group of rats was treated with 4 units of protamine–zinc insulin every 24 h for 8 days, beginning as soon as glycosuria was evident. In another group treatment with 6–8 units of protamine–zinc insulin each day was started after 25 days of diabetes and then continued for 13 days. With these insulin doses most but not all of the animals in both groups could be kept free of glycosuria throughout the treatment period. Finally the kidneys were studied from a group of 13 months rats, either non-diabetic or after 8 days of diabetes, to see if age influenced the renal response to diabetes.

At the end of the experimental period the rats were anaesthetized with pentobarbital (40 mg/kg). After clamping of the renal pedicle the left kidney was excised, immediately cooled in ice-cold sodium chloride solution (0.154 mol/l), trimmed of fat and capsule, blotted dry, weighed and homogenized in approximately 10 ml of ice-cold water. A sample of the right kidney was weighed and then dried to constant weight at 120°C to determine the water content, the remainder being examined histologically.

The kidney homogenate was made up to 20 ml in a volumetric flask, and aliquots of 5 ml were stored at −20°C. Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) were extracted from the homogenates by the Schmidt–Tannhäuser method (Munro & Fleck, 1966). RNA was determined by ultraviolet spectrophotometry at 260 nm (it was assumed that an absorbence of 1.000 corresponds to 32 µg of RNA/ml), and DNA with diphenylamine (Burton, 1956). All RNA and DNA determinations were performed in triplicate on three different 5 ml aliquots, and the mean of the three results was used. The recovery of added yeast RNA and of calf thymus DNA was over 90%. The inter-assay coefficient of variation based on twelve consecutive triplicate determinations was 3.1% for RNA and 5.5% for DNA. The protein concentration of the kidney homogenate was determined with bovine albumin as a standard (Lowry, Rosebrough, Farr & Randall, 1951).

Results

The water content of the kidneys (77–78%) was the same in all the animals studied. A 15% increase in kidney weight was evident after 3 days of diabetes (Fig. 1). Kidney growth continued, so that after 6 weeks the kidney was 90% heavier than in the control rats, whereas there was no significant alteration in kidney weight in normal rats, despite an increase in their body weight.

In the long-term experiments (Table 1) there was a steady increase in cell size, as reflected by the protein/DNA ratio, which after 42 days of diabetes was increased by 21%. The protein content of the kidney rose in parallel with the wet weight, showing a significant increase after 3 days of diabetes. The RNA content also rose within 3 days, whereas DNA was unchanged at that time but increased later. The average RNA content per cell (RNA/DNA ratio) was increased after 3 days, reaching a maximum after 8–12 days and then declining towards the end of the experimental period. The increase in average cell size (protein/DNA) was not significantly demonstrable until 8 days after the induction of the diabetes. After 42 days the kidney weight had increased by 90%. Increased cell size accounts for only a part of this as the number of cells as reflected by DNA content was increased by only 50% at that time.

The short-term experiments (Table 2) indicated that the increased kidney weight was present 36 h after the onset of glycosuria. A statistically significant increase in RNA content was demonstrable at the same time. As in the long-term experiment there was no rise in the DNA content during the first 3 days of diabetes.
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### TABLE 1. Kidney weight and content of protein, RNA and DNA after various periods of diabetes

All values refer to the left kidney alone. Results are mean values ± SEM.

<table>
<thead>
<tr>
<th>Duration of diabetes (days)</th>
<th>n</th>
<th>Body wt. (g)</th>
<th>Kidney wt. (mg)</th>
<th>Protein (mg)</th>
<th>RNA (mg)</th>
<th>DNA (mg)</th>
<th>Protein/DNA (mg/mg)</th>
<th>RNA/DNA (mg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6</td>
<td>193±1</td>
<td>574±18</td>
<td>78±2</td>
<td>2:32±0.10</td>
<td>2:92±0.06</td>
<td>26:8±0.6</td>
<td>0.79±0.02</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>175±2</td>
<td>683±11</td>
<td>83±2</td>
<td>2:69±0.08</td>
<td>3:08±0.06</td>
<td>26:7±0.6</td>
<td>0.87±0.03</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>178±3</td>
<td>796±22</td>
<td>98±4</td>
<td>3:23±0.11</td>
<td>3:42±0.07</td>
<td>28:9±0.5</td>
<td>0.94±0.02</td>
</tr>
<tr>
<td>12</td>
<td>7</td>
<td>182±3</td>
<td>919±29</td>
<td>108±4</td>
<td>3:64±0.16</td>
<td>3:83±0.11</td>
<td>28:2±0.4</td>
<td>0.94±0.02</td>
</tr>
<tr>
<td>42</td>
<td>6</td>
<td>190±5</td>
<td>1078±55</td>
<td>137±8</td>
<td>3:79±0.40</td>
<td>4:33±0.16</td>
<td>32:1±0.8</td>
<td>0.89±0.02</td>
</tr>
</tbody>
</table>

**Significance of differences**

Day 3 v. day 0: 0.00004 0.07 0.0002 0.09 0.9 0.05

Day 8 v. day 0: 0.00001 0.001 0.00009 0.00002 0.02 0.03

### TABLE 2. Kidney weight and content of protein, RNA and DNA in short-term experiments

Control rats were not given streptozotocin. All values refer to the left kidney alone. Results are mean values ± SEM.

<table>
<thead>
<tr>
<th>Time after onset of glycosuria (h)</th>
<th>n</th>
<th>Body wt. (g)</th>
<th>Kidney wt. (mg)</th>
<th>Protein (mg)</th>
<th>RNA (mg)</th>
<th>DNA (mg)</th>
<th>Protein/DNA (mg/mg)</th>
<th>RNA/DNA (mg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>171±4</td>
<td>542±15</td>
<td>76±2</td>
<td>2:61±0.07</td>
<td>3:07±0.07</td>
<td>24:8±0.48</td>
<td>0.85±0.02</td>
</tr>
<tr>
<td>0</td>
<td>6</td>
<td>174±2</td>
<td>553±14</td>
<td>80±3</td>
<td>2:67±0.09</td>
<td>3:20±0.19</td>
<td>25:0±0.73</td>
<td>0.84±0.02</td>
</tr>
<tr>
<td>12</td>
<td>6</td>
<td>169±2</td>
<td>562±20</td>
<td>86±2</td>
<td>2:82±0.04</td>
<td>3:17±0.06</td>
<td>27:0±0.76</td>
<td>0.89±0.02</td>
</tr>
<tr>
<td>24</td>
<td>6</td>
<td>167±3</td>
<td>574±25</td>
<td>84±3</td>
<td>2:86±0.06</td>
<td>3:04±0.16</td>
<td>28:1±0.76</td>
<td>0.96±0.04</td>
</tr>
<tr>
<td>36</td>
<td>6</td>
<td>164±2</td>
<td>606±13</td>
<td>93±2</td>
<td>3:12±0.07</td>
<td>3:12±0.08</td>
<td>30:0±0.50</td>
<td>1.01±0.02</td>
</tr>
<tr>
<td>48</td>
<td>6</td>
<td>162±2</td>
<td>593±13</td>
<td>94±1</td>
<td>3:13±0.04</td>
<td>3:07±0.05</td>
<td>30:6±0.37</td>
<td>1.02±0.02</td>
</tr>
<tr>
<td>60</td>
<td>6</td>
<td>168±2</td>
<td>651±13</td>
<td>97±3</td>
<td>3:20±0.10</td>
<td>3:17±0.13</td>
<td>30:6±0.77</td>
<td>1.01±0.02</td>
</tr>
</tbody>
</table>

**Significance of differences**

Control v. 0 h: 0.05 0.3 0.6 0.5 0.8 0.7

24 h v. 0 h: 0.05 0.4 0.1 0.5 0.01 0.02

36 h v. 0 h: 0.02 0.005 0.003 0.06 0.00002 0.00001

### TABLE 3. Effect of insulin treatment on kidney weight and content of protein, RNA and DNA in diabetic rats

All values refer to the left kidney alone. Results are mean values ± SEM.

<table>
<thead>
<tr>
<th>Duration of diabetes (days)</th>
<th>Time on insulin (days)</th>
<th>n</th>
<th>Body wt. (g)</th>
<th>Kidney wt. (mg)</th>
<th>Protein (mg)</th>
<th>RNA (mg)</th>
<th>DNA (mg)</th>
<th>Protein/DNA (mg/mg)</th>
<th>RNA/DNA (mg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>—</td>
<td>6</td>
<td>193±1</td>
<td>574±18</td>
<td>78±2</td>
<td>2:32±0.10</td>
<td>2:92±0.06</td>
<td>26:8±0.6</td>
<td>0.79±0.02</td>
</tr>
<tr>
<td>8</td>
<td>—</td>
<td>7</td>
<td>178±3</td>
<td>796±22</td>
<td>98±4</td>
<td>3:23±0.11</td>
<td>3:42±0.07</td>
<td>28:9±0.5</td>
<td>0.94±0.02</td>
</tr>
<tr>
<td>8</td>
<td>1–8</td>
<td>7</td>
<td>203±3</td>
<td>649±15</td>
<td>86±2</td>
<td>2:89±0.06</td>
<td>3:33±0.08</td>
<td>24:4±0.7</td>
<td>0.82±0.02</td>
</tr>
</tbody>
</table>

**Significance of differences**

Treated v. untreated (8 days): 0.00001 0.03 0.02 0.3 0.00002 0.00001

25                             | —                      | 6 | 165±8      | 964±31       | 119±4       | 3:15±0.13 | 4:33±0.20 | 27:7±0.6           | 0.73±0.02     |
| 38                             | —                      | 8 | 164±8      | 887±42       | 121±5       | 3:06±0.12 | 4:19±0.19 | 28:9±0.7           | 0.73±0.02     |
| 38                             | 25–38                  | 7 | 216±5      | 796±35       | 112±4       | 3:20±0.12 | 4:27±0.19 | 26:3±0.6           | 0.75±0.02     |

**Significance of differences**

Treated v. untreated (38 days): 0.01 0.2 0.4 0.8 0.02 0.5
days of diabetes, but in contrast to the findings described above, the protein/DNA ratio was increased by 25% after 60 h. The RNA/DNA ratio also increased much faster in this experiment.

Kidney growth was to a large extent prevented by early insulin treatment (Table 3, Fig. 1). This was, however, almost exclusively due to a prevention of cell growth (protein/DNA ratio), whereas cell number (DNA content) increased as fast as in the untreated animals. When insulin was started after 25 days of uncontrolled diabetes, a slight regression in kidney weight occurred. In this experiment DNA and RNA content were of the same magnitude in treated and untreated rats, so again cell size, but not cell number, diminished.

Table 4 shows that kidney growth was as rapid in 1 year rats as in 3 months rats. The old animals also showed an increase in protein content, RNA, DNA and protein/DNA ratio.

Discussion

It has been noted previously that diabetic rats have large kidneys, but this escaped systematic investigation until the demonstration (Ross & Goldman, 1971) that kidney weight increases by 15% during 4 days of diabetes, a similar growth to that described here. Furthermore, these authors showed that compensatory renal hypertrophy is augmented in diabetic rats. Levin, Cortes, Silveira & Rubenstein (1975) found an increased RNA/DNA ratio in diabetic rats, but do not give the duration of diabetes in their experiments. These present studies demonstrate that an increase in kidney weight occurs after 36 h of diabetes, and increased protein synthesis is indicated after only 24 h, even by such a coarse measurement as the RNA/DNA ratio. This agrees with the findings of Peterson, Greene & Reaven (1971), that an increased incorporation of amino acid into protein by kidney ribosomes from diabetic rats occurs 24 h after the injection of anti-insulin serum, or 72 h after the injection of alloxan or streptozotocin.

It is natural to compare diabetic renal hypertrophy with the compensatory hypertrophy after unilateral nephrectomy, the increase in kidney mass being approximately the same in the two conditions. Thus Kurnick & Lindsay (1968) found that after unilateral nephrectomy in rats the remaining kidney increased by 10% after 3 days and by 30% after 9 days. The corresponding figures in the present study are 15% and 30%. It is also well established that RNA accretion is detectable very early after unilateral nephrectomy, whereas DNA accumulates more slowly. The relative influence of hypertrophy and hyperplasia on kidney growth may, however, be different in the two conditions. Kurnick & Lindsay (1968) found a mere 10% increase in DNA content at a time when renal weight had increased by 40%, and Johnson & Roman (1966) calculated that in mice, after unilateral nephrectomy, hyperplasia accounts for only 25% of the renal growth. I find in these diabetic rats that the increase in DNA content parallels the kidney growth after 3 days, and that the average cell size, as reflected by the protein/DNA ratio, rises much less.

There is a discrepancy between the results of the long-term and the short-term studies. In the short term the protein/DNA ratio rose after only 24 h, but in the long-term studies this ratio was still unchanged after 3 days. The activity of protein synthesis is also increased much faster in the short-term than in the long-term experiments. This discrepancy may arise from differences in nutrition. In the short-term experiments the rats were treated with insulin for 3 days between the streptozotocin injection and the start of the measurements, and these rats did not lose weight initially. In contrast the rats in the long-term experiment showed a marked weight loss during the first 3 days. Compensatory renal hypertrophy is
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known to be depressed in starved animals (Halliburton & Thomson, 1967). The combined result of the two experiments seems to be that the diabetic kidney growth starts by hypertrophy, but later is mostly characterized by hyperplasia.

The prevention of kidney growth by treatment with insulin excludes streptozotocin per se as a causal factor. In clinical studies Mogensen & Andersen (1975) were able to reduce kidney size to normal in diabetic patients by strict insulin treatment. In rats which had been diabetic for 20 days, insulin treatment resulted in only a slight reduction of kidney size, which may result from difficulty in achieving normoglycaemia in rats with established experimental diabetes.

The cause of the kidney growth was not elucidated by this study. Many other tissues show decreased protein synthesis in diabetes (Pain & Garlick, 1974), whereas the kidney is able to double its weight during this generally wasting condition. In human diabetes mellitus plasma growth hormone is elevated (Hansen & Johansen, 1970), and this has been suggested as the growth-promoting factor for the kidney (Hansen & Mogensen, 1972), but my own unpublished studies suggest that in diabetic rats the plasma growth hormone concentration is normal.

Compensatory renal growth may represent a workload hypertrophy (Johnson, 1969), for it is evident that the energy consumption of the diabetic kidney is increased, from glucose reabsorption and gluconeogenesis. The importance of this phenomenon remains to be investigated.

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References


