Nephropathy in Sucrose-fed Rats
Electron and Light Microscopic Studies

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SUMMARY
Diffuse intercapillary glomerulosclerosis and exudative glomerulosclerosis were produced in rats fed high-sucrose diet for periods of nine to eighteen months. These lesions were observed by light and electron microscopy. Impairment of glucose tolerance was observed in the sucrose-fed animals and marked proteinuria developed in the course of long-term sucrose feeding. Such lesions did not develop in a control group of age-matched fed high-starch diets.

A high-sucrose diet results in impairment of carbohydrate tolerance in man and in the rat. A dietary survey has shown that new immigrant Yemenites to Israel consumed no sucrose while in Yemen whereas Yemenites living in Israel consume large amounts of sucrose equal to that consumed by the Western Jew. We have suggested that this difference may be one of the reasons for the greater prevalence of diabetes in the old Yemenite settler and in the general population compared to that observed in the new immigrant Yemenite.

The effects of high-sucrose and high-starch diet were compared in rats and it was found that the animals fed a high-sucrose diet for several months showed impaired rate of growth, an impairment of glucose tolerance and an increase in the amount and synthesis of liver lipids. An increased amount of soluble collagen and a reduced insoluble/soluble collagen ratio were found in the aorta.

In a preliminary report we have described diffuse intercapillary glomerulosclerosis in the kidneys of sucrose-fed rats. The present paper reports the electron and light microscopic renal changes in a larger group of such animals.

MATERIAL AND METHODS
Two groups of male albino rats of Sabra HUS* weighing 60 to 70 gm., were fed synthetic diets, containing casein 18 per cent, butter 5 per cent, salt mixture U.S.P. No. II 5 per cent and carbohydrate 72 per cent, ad libitum for periods of nine to eighteen months. The carbohydrate was given to the experimental group as sucrose, while the control group received cornstarch. Vitamins of the B group were added as a dry mix, and fat soluble vitamins were given twice weekly.

Glucose tolerance tests were performed at two to three monthly intervals, starting two months after the rats were put on the synthetic diets. The animals were fasted for sixteen hours and given 350 mg. glucose per 100 gm. body weight in 35 per cent solution by gastric tube. At 0, 60 and 120 min. following the glucose load, duplicate samples of 0.1 ml. of blood were taken from the tip of the tail, and blood glucose was determined by the Somogyi/Nelson method.

The urine was collected in a metabolic cage overnight, urinary glucose was determined by Tes-Tape, and the protein content by the Kingsbury-Klark method. At the appropriate period twenty-nine starch-fed and thirty-one sucrose-fed rats were anesthetized by ether and bled. The kidneys were removed and weighed (table 1) and specimens fixed in 10 per cent neutral formalin for light microscopy. These were embedded in paraffin and sections 3 to 4 μ thick were stained with hematoxylin and eosin (H & E), period-acid Schiff (PAS), Ritter-Olsen method (PAS-colloidal iron), Masson's trichrome and silver methenamine method. Frozen sections were cut by cryostat and stained with Sudan III for lipids.

For electron microscopy small blocks of kidney tissue were cut while immersed in a cold solution of 4 per cent paraformaldehyde w/v 0.5 per cent glutaraldehyde v/v in Millonig's phosphate buffer (pH 7.4) for one

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*Hebrew University strain.
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TABLE 1

The glucose tolerance, body weight, and kidney weight in sucrose and starch-fed animals

<table>
<thead>
<tr>
<th>Months on diet</th>
<th>Diet</th>
<th>No. of animals</th>
<th>0 min.</th>
<th>Glucose tolerance (*100 ηg/kg)</th>
<th>120 min.</th>
<th>Body weight (100 gm. b.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>Starch</td>
<td>7</td>
<td>71 ± 2.1</td>
<td>112 ± 4.6</td>
<td>108 ± 6.9</td>
<td>442 ± 22</td>
</tr>
<tr>
<td></td>
<td>Sucrose</td>
<td>5</td>
<td>82 ± 3.0</td>
<td>140 ± 5.3</td>
<td>138 ± 4.9</td>
<td>330 ± 11</td>
</tr>
<tr>
<td>16</td>
<td>Starch</td>
<td>5</td>
<td>70 ± 1.9</td>
<td>108 ± 2.3</td>
<td>102 ± 5.3</td>
<td>525 ± 95</td>
</tr>
<tr>
<td></td>
<td>Sucrose</td>
<td>9</td>
<td>75 ± 2.8</td>
<td>142 ± 4.1</td>
<td>135 ± 5.1</td>
<td>486 ± 15</td>
</tr>
<tr>
<td>12</td>
<td>Starch</td>
<td>9</td>
<td>65 ± 1.7</td>
<td>105 ± 3.5</td>
<td>100 ± 4.6</td>
<td>501 ± 18</td>
</tr>
<tr>
<td></td>
<td>Sucrose</td>
<td>8</td>
<td>72 ± 2.0</td>
<td>140 ± 5.0</td>
<td>136 ± 5.0</td>
<td>387 ± 12</td>
</tr>
<tr>
<td>9</td>
<td>Starch</td>
<td>8</td>
<td>69 ± 2.0</td>
<td>107 ± 2.8</td>
<td>105 ± 3.8</td>
<td>447 ± 18</td>
</tr>
<tr>
<td></td>
<td>Sucrose</td>
<td>9</td>
<td>70 ± 2.3</td>
<td>135 ± 4.6</td>
<td>140 ± 6.1</td>
<td>352 ± 13</td>
</tr>
</tbody>
</table>

* Sucrose versus starch
† P < 0.05

RESULTS

The body weight, glucose tolerance, weight of the right kidney and the number of rats with pathological changes in the kidneys in the experimental and control groups are shown in Table 1.

The body weight of the sucrose-fed animals was less than that of the starch-fed animals. Glucose tolerance was impaired in the sucrose-fed animals. The starch-fed rats and the sucrose-fed rats with mild or no pathological changes in the kidneys had proteinuria ranging from 2 to 9 mg./24 hr. whereas the sucrose-fed animals with renal pathological changes had more marked proteinuria ranging from 12 to 45 mg./24 hr.

No renal pathology was evident in twenty-one starch-fed and in ten sucrose-fed animals (Table 2). In eight out of twenty-nine starch-fed and in seven out of thirty-one sucrose-fed rats, mild renal lesions were noted. These
lesions were focal and were present in only 5 to 10 per cent of the glomeruli. They consisted of glomerular obsolescence and sclerosis, the glomerular tufts being shrunken and the renal corpuscle diminished in size (figure 1). Periglomerular fibrosis was also noted.

In fourteen out of thirty-one sucrose-fed rats conspicuous pathological changes were found in the kidneys. In these animals the kidneys were usually enlarged, weighing up to 4 gm. in extreme cases, and in some a yellowish discoloration of the parenchyma was noted. The outer surface was finely granular. In ten of the fourteen the pathological changes were moderately severe. The lesions were diffuse and generalized and in nine of them more than 90 per cent of the glomeruli were involved. The lesions consisted of enlargement of the glomerular tufts, hypocellularity and a marked increase of the mesangial areas (figure 2), especially as demonstrated by the PAS and silver methenamine methods. The glomerular capillary wall, in most cases, appeared of normal thickness but an irregular segmental thickening was occasionally noted. Bowman’s capsule was occasionally slightly thickened. Four of the fourteen animals with renal lesions showed more severe glomerular changes. In addition to the diffuse mesangial increase, a marked thickening of the capillary wall was noted (figure 3). In many of these glomeruli smudgy eosinophilic vacuolated nodules were present in the wall of the capillary, which partially obliterated the lumen (figure 4). These lesions stained red with the Masson’s trichrome stain and were also positive with Sudan stain for lipids, as in the case of the classical exudative lipo-hyaline lesion. No sudanophilic droplets were observed in glomerular lesions other than in the exudative type. In the majority of the animals no tubular or interstitial lesions were present. Focal tubular atrophy, dilated tubules containing eosinophilic casts and foci of a chronic interstitial inflammatory infiltrate were, however, present in association with the more severe glomerular changes. Blood vessel changes were
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FIG. 4.
Sucrose-fed rat. Severe glomerular lesion. Enlarged glomerulus with mesangial increase, capillary wall thickening and exudative lesions (arrows). Hematoxylin and eosin X 420.

noted inconsistently; no changes were present in the arcuate arteries. However, in five instances the intralobular arteries were markedly thickened with luminal narrowing and in three animals marked arteriolar sclerosis was noted. In sections stained with Masson's trichrome stain the thickening was seen to be due mainly to medial hypertrophy, and no PAS positive material was found in the vessel walls. There was no correlation between the severity of the vascular and glomerular changes.

Electron microscopic studies of the kidneys were performed in seven starch-fed rats (six for eighteen months and one for sixteen months) and in nine sucrose-fed rats (five for eighteen months, three for sixteen months and one for twelve months). Three or more glomeruli were studied in each case. Tubules and vessels were not studied. In general the electron microscopic findings confirmed the light microscopic findings. In the starch-fed group no glomerular pathology was noted. Of the sucrose-fed rats examined by the electron microscope, seven had moderate and two had severe glomerular lesions, on light microscopy. In the sucrose-fed animals the ultrastructural changes were as follows: the peripheral basement membrane was irregularly thickened, revealing striking variations: the thickness of the basement membrane in the starch-fed group varied from 1,500 to 4,000 Å the average thickness being 3,050 Å ± 91 whereas in the sucrose-fed group it varied from 2,140 to 6,020 Å with an average of 3,990 ± 102 (table 3). The difference between the two groups is statistically significant p < 0.05. The epithelial and endothelial sides of the basement membrane were of a regular contour. There was a marked increase of the mesangial matrix, the electron density of which was the same as that of the peripheral basement membrane (figure 5). Occasionally an increase in the number of mesangial cells was observed. In a single mesangial area in one animal, coarse fibrils, probably collagen fibrils, were present within the mesangial matrix. Between the basement membrane branches many islands of mesangial cell cytoplasm were present. The visceral epithelial cells were swollen and their cytoplasm contained numerous inclusions, consisting mainly of lipid droplets and dense bodies, which most probably represent hyalin droplets. The foot processes of the visceral epithelial cells were mostly discrete and regularly applied on the epithelial side of the basement membrane. Fusion of foot processes was noted in the more severe glomerular lesions. The exudative lesion consisted of finely granular electron dense material (fibrinoid) located between the basement membrane and the endothelial cell (figure 6). In most of these lesions the endothelial cell cytoplasm beneath this electron dense material was swollen and contained a large number of lipid droplets (figure 7) and sometimes also clefts of cholesterol crystals. The lipid droplets were seen only in the exudative lesions and were frequent in the four rats with the severe glomerular lesions.

DISCUSSION

Long-term feeding with a high-sucrose diet for nine to eighteen months has resulted in impaired glucose tolerance and in renal disease characterized by severe proteinuria and morphological changes of diffuse inter-
TABLE 3
Glomerular basement membrane thickness (Å)

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Age</th>
<th>Diet</th>
<th>Mean ± S.E.M.</th>
<th>Range</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>Sucrose</td>
<td>3,770 ± 300</td>
<td>2,140-5,710</td>
<td>N.S.*</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td></td>
<td>3,910 ± 115</td>
<td>2,860-5,000</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td></td>
<td>3,240 ± 150</td>
<td>2,670-4,290</td>
<td>N.S.</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td></td>
<td>4,410 ± 158</td>
<td>3,330-5,000</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td></td>
<td>4,480 ± 240</td>
<td>2,780-6,110</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>6</td>
<td>18</td>
<td></td>
<td>4,010 ± 160</td>
<td>2,602-5,890</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>7</td>
<td>16</td>
<td></td>
<td>3,980 ± 180</td>
<td>2,912-6,020</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Average</td>
<td>3,990 ± 102</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>18</td>
<td>Starch</td>
<td>3,470 ± 85</td>
<td>2,670-4,000</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>18</td>
<td></td>
<td>3,250 ± 117</td>
<td>2,380-3,970</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>16</td>
<td></td>
<td>3,220 ± 131</td>
<td>1,500-3,000</td>
<td></td>
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<tr>
<td>11</td>
<td>18</td>
<td></td>
<td>1,800 ± 152</td>
<td>2,670-4,000</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>18</td>
<td></td>
<td>3,520 ± 107</td>
<td>2,451-3,600</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Average</td>
<td>3,050 ± 91</td>
<td></td>
<td>&lt; 0.05†</td>
</tr>
</tbody>
</table>

* Statistical difference from animal No. 12 with the greatest basement membrane thickness among the starch-fed animals.
† Between averages of means of starch and sucrose-fed animals.

FIG. 5. Electron micrograph of the glomerular mesangium (Mes.) in the moderate lesion. Note the increase of the mesangial matrix, the electron density of which is identical to the peripheral basement membrane. Many islands of cytoplasmic processes of the mesangial cell are present with the mesangial matrix. Note the normal distribution of the epithelial cell (Ep.) foot processes X 22,800.
capillary glomerulosclerosis. None of the control age-matched rats fed a high-starch diet for the same period showed these changes. The pathological changes were already observed after nine months of feeding, i.e., the shortest period of sucrose feeding after which the animals were examined in this study. No correlation was found between the severity of the renal lesion and the duration of the feeding period. In eight starch-fed rats aged nine to eighteen months, mild glomerulosclerotic lesions were observed. These lesions were focal and involved 5 to 10 per cent of the glomeruli. The renal corpuscles were smaller in size and glomerular tuft was shrunken. Similar lesions were also seen in seven of the sucrose-fed rats of the same ages. These renal lesions are similar to those described in aged rats.\textsuperscript{11,12}

Lesions of different nature and extent were observed in fourteen of thirty-one sucrose-fed rats, and in none of the twenty-nine starch-fed age-matched control rats.

The morphological renal lesions observed in these animals by light and EM, closely resemble those observed in experimental diabetes in the rat\textsuperscript{13-16} in both spontaneous,\textsuperscript{17,18} and experimentally induced diabetes in other animals\textsuperscript{19,20} and in human diabetic glomerulosclerosis.\textsuperscript{21,22} The lesion observed in the present study was mainly a diffuse intercapillary glomerulosclerosis and in the more advanced cases exudative lesions appeared. The exudative lesions differ somewhat from those in human lesions. The fat droplets are located in the cytoplasm of the endothelial cell adjacent to the fibrinoid, whereas in the classical human exudative lesions, fat
droplets are extracellular and are present within the fibrinoid material. It is possible that our findings represent an earlier stage in the formation of the exudative lesion. As in other experimentally induced diabetic rats, nodular intercapillary glomerulosclerosis was not observed; moon crater-like formations of the type observed in alloxan diabetic rats were not seen in the present experiment.

The arterial and arteriolar sclerosis that accompanied some of the lesions is noteworthy. In the present study this thickening was due to medial hypertrophy and the lesions differed from the classical human diabetic vascular lesion in which PAS positive material accumulates.

The results of the present experiment indicate that even in the presence of a "slight" impairment of carbohydrate metabolism renal glomerular lesions develop. The impairment of carbohydrate metabolism in our animals was mild and can be referred to as a latent diabetic stage. The fact that not all animals fed sucrose for the same period of time developed renal lesions indicate that there is also an individual sensitivity to a change in nutrition.

ACKNOWLEDGMENT

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REFERENCES


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