Arteriolar Anatomical and Functional Abnormalities in Juvenile Mice with Genetic or Streptozotocin-Induced Diabetes Mellitus

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SUMMARY We tested the hypothesis that a state of hyperglycemia in hyper- and hypoinsulinemic animal models of diabetes mellitus is associated with arteriolar pathology. Hyperglycemic, hyperinsulinemic obese mice and Jackson diabetic mice at age 8 weeks were used as hyperglycemic, hyperinsulinemic models; and normal mice, treated with streptozotocin (100 mg/kg) at age 4 weeks and studied at age 8 and 16 weeks, served as hyperglycemic, hypoinsulinemic models. All mice were anesthetized lightly, and the microvasculature of the cremaster muscle was observed in vivo. The inner diameters of the large arterioles in diabetic mice are significantly (P < 0.06) smaller than normal, yet the smallest arterioles in diabetic mice have significantly (P < 0.05) increased inner diameters. The cross-sectional areas of the vessel walls for large and intermediate diameter arterioles are significantly (P < 0.05) decreased by as much as 50% in diabetic mice. The smallest arterioles have an increased wall area but have a poorly developed coat of vascular smooth muscle cells. The number of arterioles open to blood flow is significantly (P < 0.05) decreased by 20-50% in all diabetic mice in the intact and passive (sodium nitroprusside suffusion, 1 mg/1 ml) state. The dilation of the majority of arterioles from the intact to passive state is significantly (P < 0.05) less than normal in diabetics. The data indicate a common arteriolar pathology in hyperglycemic mice that are either hyper- or hypoinsulinemic. The decreased number of arterioles and the failure of arterioles to dilate properly is attributed to an abnormal development of the vessel wall in juvenile diabetic mice. Cirr Res 45: 390-396, 1979

THE development of microangiopathy in diabetes mellitus has been attributed to such diverse causes as excessive growth hormone (Ditzel, 1975; Lundbaek et al., 1971), hyperglycemia (Beauchemin et al., 1975), and insulin deficiency (Ditzel, 1975), among other factors (Ditzel et al., 1958). However, neither the exact cause or causes of the angiopathy nor the major initial vascular lesion precipitated by the as-yet unknown mechanism have been defined. The problem to be resolved is, therefore, 2-fold in that the primary lesion in angiopathy must be described before the basic cause of the lesion can be resolved. At first approximation, one would assume that the many descriptions of diabetic angiopathy, in particular the capillary basement membrane anomalies (Beauchemin et al., 1975; Handelsman et al., 1962; Parving et al., 1976) in humans and animals, would predict that major vascular disturbance. However, in the majority of studies, diabetes is well established before the vasculature is observed. Consequently, there is a distinct possibility that the initial lesion, and cause of the lesion, in diabetic angiopathy is masked by partial compensation, as well as secondary pathology, if the vasculature is not studied as early as possible.

If diabetic angiopathy occurred in only hyperglycemic animals with deficient amounts or activities of insulin, the study of angiopathy would be facilitated. However, Beauchemin et al. (1975) report that hyperglycemic mice with increased or decreased plasma insulin concentrations have abnormally thickened capillary basement membranes. In addition, Papachristodoulou et al. (1976) report that

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similar types of retinal angiopathy occur in both normal rats fed on a sucrose diet and rats made diabetic with streptozotocin. There is, therefore, a distinct possibility that conditions that exist during hyperglycemia of any origin are directly or indirectly correlated to the development of angiopathy. However, there are insufficient data available to predict whether equivalent types of angiopathy, other than a thickened capillary basement membrane and generalized vascular deterioration, occur in all forms of hyperglycemia. Therefore, the present study was designed to evaluate and compare the early stages of arteriolar angiopathy in hyperglycemic juvenile mice with either increased or decreased plasma insulin concentrations. The mice studied were the hyperglycemic, hyperinsulinemic obese (Ob/Ob) mouse, the Jackson diabetic (Db/Db) mouse (Coleman, 1978; Wyse and Dulin, 1970), and normal mice treated with streptozotocin, which are hyperglycemic, hypoinsulinemic (Junod et al., 1969) models of diabetes. We measured the diameters, wall thicknesses, and number of vessels open to blood flow in the skeletal muscle vasculature of normal and hyperglycemic mice. In addition, the ability of arterioles to dilate or open, if normally closed, was documented as all vascular control was abolished. The data were used to determine if a common early microvascular lesion occurred in hyperglycemia of different origins in diabetic mice.

Methods

Jackson diabetic mice (Db/Db) (C57BL/KSJ-Db) and obese mice (Ob/Ob) (C57BL/6J-ob) were studied at age 8-10 weeks. The characteristics of the Db/Db and Ob/Ob mice have been reviewed recently (Coleman, 1978; Wyse and Dulin, 1970). In addition, normal mice treated at age 4-6 weeks with streptozotocin (STZ), 100 mg/kg (Junod et al., 1969), were studied at age 8-10 weeks (STZ-8) and age 16-18 weeks (STZ-16). Eight to 10-week-old normal Swiss-Webster mice and control nondiabetic mice (litter or colony mates) of the Db/Db strain were used together as controls because no significant difference in vascular characteristics could be detected with the protocol used.

All mice were anesthetized with a saline solution of 2% α-chloralose and 10% urethane. An initial anesthetic solution of 0.5 ml was given per 100 g of body weight, and supplemental doses of 0.2 ml/100 g were administered, as needed, to maintain light surgical depth anesthesia. The trachea was intubated to maintain a patent airway. The mouse’s core temperature was held at 37°C by a heating mat.

The cremasteric muscle was prepared for observation with a modification of the Baez and Orkin (1967) technique. In brief, the anterior surface of the left scrotum was slit, and the sac-like cremasteric muscle was dissected free of the scrotal skin. The cremasteric muscle then was slit along the anterior surface and spread over a circular translucent pedestal (1 cm diameter). A cover glass was placed over the tissue and the cover glass, tissue, and pedestal were sealed together with petroleum jelly to prevent dehydration and contamination by atmospheric oxygen. The tissue and pedestal were heated to 34-35°C, which is the in situ temperature of the scrotum as measured in conscious mice. Throughout the period of surgery, the cremasteric muscle was irrigated with a warm physiological solution (Normosol-R, Abbott Labs). The preparations were allowed to equilibrate for 30 minutes after completion of the surgery. Preparations were rejected if: (1) arterial or venous blood flow was impaired, or (2) hemorrhage or red blood cell extravasation was present. The success rate for normal mice was 70-75% and for diabetic mice, 50-55%. The number of mice successfully studied is as follows: normal mice (NM), 17; Ob/Ob, 7; Db/Db, 6; STZ-8, 12; and STZ-16, 8.

Measurement Techniques

The cremaster muscle was transilluminated and observed with a Wild M-20 microscope. A closed circuit video system was used to display and simultaneously record images. The magnification factors were determined by measurement of a micrometer, marked in 10 μ units and ranged from 500 to 1600× during the majority of experiments.

Vessel Classification

Arterioles were identified on the basis of their branching sequence, using a modification of a system proposed by Wiedeman (1968). The largest arterioles to enter the tissue are termed first order arterioles (1A). Subsequent branches were identified as second (2A), third (3A), and fourth (4A) order arterioles. In general, a new order vessel was designated if the new vessel in question branched from the parent vessel at an angle near 90°. If a parent vessel simply bifurcated and each branch had essentially the same diameter as the parent vessel, the branches were considered to be the same branch order as the parent vessel. All mice studied had four basic branch orders of arterioles on the scheme presented.

In determining the total number of arterioles of a given type in the tissues, we counted a vessel which bifurcated into two similar order vessels as a single vessel. This approach was used because vessels seldom bifurcated until they reached near their termination. The branches were sufficiently short that they contributed very little to overall tissue perfusion compared to the parent vessel. Therefore, the terminal branches of a vessel were excluded in the counting of vessels and did not bias the count of the number of vessels in a given classification order.

Measurement Criteria

The inner vessel wall was defined as the point of junction of the plasma sleeve around the red blood
cell column and a continuous line, which is considered to be the innermost edge of the endothelial lining of the vessel. The outermost edge of the vessel wall was considered to be the outer edge of vascular smooth muscle cells. If the vessel wall thickness was not equal in width on both sides of the vessel, the point of measurement was moved slightly until both walls were equal in width. In all cases, the widest possible width of the wall was measured.

**Protocol**

The numbers of vessels in each classification order were counted in the entire cremasteric muscle area on the pedestal (78.5 mm²). In addition, arterioles of known type were selected randomly for measurement of the inner diameter and thickness of the vessel wall on one side of the arteriole. The vessels in question were identified on a map made of the vasculature, so that these vessels could be remeasured at any time. The cover glass then was removed and the tissue irrigated with sodium nitroprusside (1 mg/ml) to abolish all vascular control and produce a passive state. The cover glass then was replaced and the diameters of vessels previously selected again were measured. In addition, the numbers of arterioles open to blood flow were counted and compared to prior measurements during the intact state. The mouse then was killed with an intracardiac injection of anesthetic solution. The muscle tissue on the pedestal was cut free of all tissues not on the pedestal, blotted, and weighed immediately.

**Data Analysis**

The mean and standard error of the mean was calculated for each variable to be reported. Duncan’s new multiple range test (Steel and Torrie, 1960) for groups with unequal numbers of observations was used to estimate significant differences between means. All data were referenced to values obtained in normal 8-10-week-old mice and nondiabetic litter or colony mates of Db/Db mice for determination of significant differences unless otherwise noted. Microvascular characteristics in normal Swiss-Webster mice and nondiabetic litter and colony mates of Db/Db mice are not detectably different, and both were considered NM. A P value of 0.05 or smaller was considered to indicate a significant difference in the means of two populations.

**Results**

**Animal Characteristics**

The age, body weight, cremasteric muscle weight, and blood glucose concentration (mg/100 ml plasma) for each mouse type are presented in Table 1. The weights of the cremasteric muscles for all mouse types, except STZ-16, are not significantly different. These weights, including those in STZ-16, represent essentially pure muscle tissue because few or, often, no fat cells were present. The blood glucose concentrations were determined in nonfasted mice. The blood glucose concentrations of all hyperglycemic mice were significantly (P < 0.01) higher than normal. In addition, glucose was found in the urine of all diabetic mice if collected over 4-hour periods. Polyuria and glucosuria were noted in STZ-treated mice within 3 days after the single injection of toxin. Measurements of mean arterial blood pressure in the mice were not made routinely due to the difficulty of arterial cannulation. However, successful pressure measurements did indicate that mean arterial pressure of 70-90 mm Hg exists in both normal and diabetic mice when anesthetized and surgically prepared for study as previously described. Measurements of mean arterial pressures in conscious mice with implanted arterial cannulas in the tail artery and advanced to the abdominal aorta were routinely in the range of 80-100 mm Hg.

**Arteriolar Diameter**

The mean inner diameters for each order of arteriole during intact conditions are presented in Table 2 for each mouse type. These data indicate three distinct situations for diameters of vessels in hyperglycemic mice. First, the diameters of the 1A and 2A of diabetic mice, with the exception of 2A in STZ-16, were significantly (P < 0.05) smaller than in normal mice. This was particularly evident for the Ob/Ob and Db/Db mice. Second, the inner

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**Table 1: Physical Characteristics of Normal and Diabetic Mice**

<table>
<thead>
<tr>
<th>Mouse type</th>
<th>Age (wks)</th>
<th>Duration of hyperglycemia (wks)</th>
<th>Body wt (g)</th>
<th>Cremaster muscle wt (mg)</th>
<th>n</th>
<th>Plasma glucose* (mg/100 ml)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM</td>
<td>8-10</td>
<td></td>
<td>28.6 ± 1.2</td>
<td>11.3 ± 0.9</td>
<td>16</td>
<td>136 ± 8</td>
<td>13</td>
</tr>
<tr>
<td>Ob/Ob</td>
<td>8-10</td>
<td>4-6</td>
<td>41.5 ± 1.2†</td>
<td>9.3 ± 0.8</td>
<td>6</td>
<td>293 ± 15†</td>
<td>6</td>
</tr>
<tr>
<td>Db/Db</td>
<td>8-10</td>
<td>4-6</td>
<td>44.2 ± 1.6†</td>
<td>10.6 ± 0.8</td>
<td>6</td>
<td>438 ± 23†</td>
<td>6</td>
</tr>
<tr>
<td>STZ-8</td>
<td>8-10</td>
<td>4-6</td>
<td>26.8 ± 1.1</td>
<td>10 ± 0.8</td>
<td>12</td>
<td>321 ± 10†</td>
<td>12</td>
</tr>
<tr>
<td>STZ-16</td>
<td>16-18</td>
<td>12-14</td>
<td>25.4 ± 1.6</td>
<td>14.1 ± 0.9†</td>
<td>8</td>
<td>500 ± 69†</td>
<td>6</td>
</tr>
</tbody>
</table>

* Measured in nonfasted mice.
† P < 0.05 compared to NM.
diameters of 3A arterioles (the intermediate-sized arterioles) were not significantly \((P > 0.05)\) different in normal and hyperglycemic mice. Finally, the diameters of 4A arterioles, the smallest type of arteriole, were significantly \((P < 0.01)\) greater in all hyperglycemic mice than in normal mice.

A major concern in regard to arteriolar diameters is the ability of the individual vessels to dilate after vascular control is abolished. Sodium nitroprusside was used to abolish vascular control. The dilation of vessels in this situation represented a nonspecific loss of both neural and local hormonal control. The percent changes in diameters from the intact to passive state are presented in Table 3.

In all mice, the 1A arterioles did not dilate significantly \((P > 0.05)\) with respect to their control diameter or exhibit a percent change in diameter different in diabetic mice than the response of 1A in NM (Table 3). The 2A arterioles of Db/Db and STZ-16 dilated significantly \((P < 0.05)\) less than in normal mice. The responses of 2A in normal, obese, and STZ-8 mice were not significantly different. In contrast, the 3A arterioles of all diabetic mice dilated by essentially normal proportions. The major dilation, 45\% of control, for the 4A arterioles in normal mice was in sharp contrast to the comparatively small dilation, 10–15\%, for 4A in all hyperglycemic mice.

### Arteriolar Wall Characteristics

The vessel wall thickness-to-lumen diameter ratio for each order of the arteriole in each type of mouse is shown in Table 2. Although the diameters of the 1A arterioles were different in the various mouse types (Table 2), the wall thickness-to-lumen diameter ratios were equal for all mice (Table 2). The same situation also existed for the 2A with the exception that the wall-to-lumen ratio was significantly \((P < 0.05)\) greater than normal in Ob/Ob. The 3A and 4A of hyperglycemic mice had significantly \((P < 0.05)\) smaller wall-to-lumen ratios than in comparable order arterioles of normal mice.

The cross-sectional area of the vessel wall was calculated for each order of arteriole in each of the mouse types. The cross-sectional vessel wall area was calculated based on the inner vessel radius, plus wall thickness, minus vessel area based on inner vessel radius. These data, presented in Table 2, indicated that a major reduction in wall materials existed for 1A, 2A, and 3A in hyperglycemic mice, except 1A in STZ-8. In contrast, the wall area of the smallest arterioles, 4A, was increased above normal for all hyperglycemic mice. However, the smooth muscle coat along the 4A and all arterioles was poorly developed compared to normal vessels, in the sense that individual smooth muscle cells were clearly outlined in normal mice but were marginally visible in the diabetic mice.

### Tissue Vascularization

Tissue vascularization for the purposes of this presentation was defined as the number of arterioles of a given order on the 78.5 mm² pedestal. The number of vessels of a given type in this area on the pedestal also closely corresponded to the number of vessels per mass of tissue because all mice, except STZ-16, had equal cremaster muscle weights (Table 1). Numbers of vessels open to blood flow,
both before and after nitroprusside application, are presented in Table 3. In some instances, vascularity did increase as vascular control was abolished, and previously closed vessels opened to flow to attain the maximum vascularity in each mouse type. The vascularity of the 1A in all mice was equal for both the intact and passive state. The number of 2A and 3A arterioles in all hyperglycemic mice, except for 3A in STZ-8 mice, was substantially depressed \((P < 0.05)\) compared to both the intact and passive state in normal mice. In addition, the number of 4A was decreased below normal in Ob/Ob and Db/Db for the passive state. The STZ-8 and STZ-16 had essentially normal numbers of 4A open to blood flow in the intact and passive states. However, the weight of the cremaster muscle in STZ-16 was more than normal by approximately 25\% (Table 1). Therefore, on a basis of numbers of vessels per mg tissue, have 20-25\% fewer than normal numbers of 3A and 4A.

### Discussion

The two major issues of this study are whether a major initial vascular lesion occurs in skeletal muscle tissue early in juvenile diabetes and if this lesion is qualitatively similar in hyperglycemics of different origin. On the basis of the data obtained and presented in Table 2, the most important finding was that the substance of the arteriolar wall, as judged by the vessel wall cross-sectional area, is markedly decreased in all except the smallest arterioles (4A) in hyperglycemic mice with either increased or decreased plasma insulin concentrations. The primary component of the arteriolar wall was vascular smooth muscle, as judged by high power (1600X light microscope) observation of the vessel wall in normal and diabetic mice. Even the smallest arterioles, the 4A, which had a significantly \((P < 0.05)\) greater wall area than normal in all diabetic mice, did not have distinctly outlined vascular muscle cells as could be readily observed in normal mice (Table 2). Therefore, we propose that improper maintenance of the vessel wall, in particular the muscle component, is an early vascular manifestation of juvenile onset diabetes in mice. The fact that all diabetic mice, whether models of hyper- or hypoinsulinemic diabetes mellitus (Coleman, 1978; Wyse and Dulin, 1970; Junod et al., 1969), exhibited qualitatively and, in many cases, quantitatively similar vascular disturbances further indicated that the existence of hyperglycemia, rather than the genetic or drug-induced form of diabetes, was related to the development of angiopathy. This is not to say that hyperglycemia of itself caused the microangiopathy but that the various models of hyperglycemia in diabetic mice were associated with equivalent forms of microangiopathy.

The existence of smaller than normal vessel wall cross-sectional areas for the majority of arterioles in diabetic mice, as indicated by the data in Table 2, may be the direct cause of other arteriolar impairments in these mice. The altered state of the vessel wall is apparently not just a problem of microvessels. Wolinsky et al. (1978) have stated that, after 4 weeks of STZ-induced diabetes in

### Table 3 Percent of Control Arteriolar Diameter and Number of Vessels Open to Blood Flow during Passive Conditions in Normal and Diabetic Juvenile Mice

<table>
<thead>
<tr>
<th>Vessel and mouse type</th>
<th>% of control diameter-passive</th>
<th>Nv</th>
<th>Control</th>
<th>Passive</th>
<th>NA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A-NM</td>
<td>98 ± 6</td>
<td>11</td>
<td>1.4 ± 0.2</td>
<td>1.4 ± 0.2</td>
<td>8</td>
</tr>
<tr>
<td>1A-Ob/Ob</td>
<td>106 ± 6</td>
<td>6</td>
<td>1.7 ± 0.2</td>
<td>1.7 ± 0.2</td>
<td>6</td>
</tr>
<tr>
<td>1A-Db/Db</td>
<td>94 ± 12</td>
<td>9</td>
<td>1.2 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>6</td>
</tr>
<tr>
<td>1A-STZ-8</td>
<td>100 ± 3</td>
<td>13</td>
<td>1.3 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>12</td>
</tr>
<tr>
<td>1A-STZ-16</td>
<td>102 ± 1</td>
<td>9</td>
<td>1.1 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>8</td>
</tr>
<tr>
<td>2A-NM</td>
<td>125 ± 9</td>
<td>20</td>
<td>13.1 ± 0.7</td>
<td>13.1 ± 0.7</td>
<td>8</td>
</tr>
<tr>
<td>2A-Ob/Ob</td>
<td>121 ± 8</td>
<td>12</td>
<td>10.7 ± 1.0*</td>
<td>10.7 ± 1.0*</td>
<td>6</td>
</tr>
<tr>
<td>2A-Db/Db</td>
<td>107 ± 5*</td>
<td>13</td>
<td>5.7 ± 1.0*</td>
<td>5.7 ± 1.0*</td>
<td>6</td>
</tr>
<tr>
<td>2A-STZ-8</td>
<td>114 ± 7*</td>
<td>28</td>
<td>10.1 ± 1.1*</td>
<td>10.1 ± 1.1*</td>
<td>12</td>
</tr>
<tr>
<td>2A-STZ-16</td>
<td>111 ± 5*</td>
<td>14</td>
<td>10.6 ± 0.7*</td>
<td>10.6 ± 0.7*</td>
<td>8</td>
</tr>
<tr>
<td>3A-NM</td>
<td>139 ± 8</td>
<td>58</td>
<td>63 ± 4*</td>
<td>67 ± 5</td>
<td>8</td>
</tr>
<tr>
<td>3A-Ob/Ob</td>
<td>126 ± 6</td>
<td>21</td>
<td>54 ± 4*</td>
<td>54 ± 4*</td>
<td>6</td>
</tr>
<tr>
<td>3A-Db/Db</td>
<td>129 ± 9</td>
<td>23</td>
<td>27 ± 4*</td>
<td>27 ± 4*</td>
<td>6</td>
</tr>
<tr>
<td>3A-STZ-8</td>
<td>144 ± 7*</td>
<td>47</td>
<td>53 ± 4*</td>
<td>52 ± 5</td>
<td>12</td>
</tr>
<tr>
<td>3A-STZ-16</td>
<td>135 ± 6</td>
<td>29</td>
<td>56 ± 3*</td>
<td>74 ± 9*</td>
<td>8</td>
</tr>
<tr>
<td>4A-NM</td>
<td>145 ± 7</td>
<td>86</td>
<td>320 ± 23</td>
<td>387 ± 27</td>
<td>17</td>
</tr>
<tr>
<td>4A-Ob/Db</td>
<td>109 ± 6*</td>
<td>42</td>
<td>290 ± 14</td>
<td>293 ± 17</td>
<td>6</td>
</tr>
<tr>
<td>4A-Db/Db</td>
<td>111 ± 5*</td>
<td>45</td>
<td>162 ± 27*</td>
<td>169 ± 22*</td>
<td>6</td>
</tr>
<tr>
<td>4A-STZ-8</td>
<td>115 ± 4*</td>
<td>89</td>
<td>294 ± 20</td>
<td>347 ± 32</td>
<td>12</td>
</tr>
<tr>
<td>4A-STZ-16</td>
<td>118 ± 3*</td>
<td>56</td>
<td>318 ± 20</td>
<td>408 ± 46</td>
<td>8</td>
</tr>
</tbody>
</table>

Data are ± SEM. Nv is number of vessels observed; NA is number of mice observed.

* Significant \((P < 0.05)\) differences from normal.

† STZ-16 have heavier than normal muscles and, on the basis of number of vessels per mg tissue, have 20-25\% fewer than normal numbers of 3A and 4A.
juvenile rats, the aorta has a thinner than normal wall, although the wall thickness approaches normal development by adult life. In addition, Wolinsky et al. (1978) have demonstrated a serious impairment of the biochemical properties in vascular smooth muscle during the diabetic state. In the context of the present study, the failure of the large arterioles, the 1A and 2A, to attain a normal internal diameter may have reflected a failure of proper growth of the vessel wall (Table 2). The absence of dilation to a normal diameter by these vessels when all vascular control was abolished (Table 2) further supports the proposal that these vessels are smaller than normal and not simply constricted during resting conditions. A second abnormality of importance was the decreased numbers of 2A, 3A, and 4A arterioles in the majority of diabetic mice. It is conceivable that, if the vessel wall either does not develop properly or fails to be properly maintained, the formation and maintenance of arterioles will be impaired, such that fewer than normal numbers of vessels are ultimately available. This proposal is based on the numbers of vessels open to blood flow after all vascular control is abolished (Table 2). Therefore, temporary closure of vessels by vasoactive mechanisms did not have a bearing on the number of vessels counted. Unfortunately, quantitative information on the number of arterioles available for blood flow is not available for human subjects. However, there are indications that either poorly developed vascular branching patterns or permanent losses of arterioles do occur in cutaneous and ocular tissues of diabetic humans (Handelsman, 1962; Banson and Lacey, 1964; Ditzel and Duckers, 1957; Ditzel and Saglid, 1954; Ditzel et al., 1958; Wallow and Engerman, 1977).

With the incidence of fewer than normal numbers of arterioles and smaller than normal diameters for the large arterioles (1A, 2A) in diabetic mice, there was undoubtedly an increase in vascular resistance of as yet undetermined magnitude. Measurements of mean arterial blood pressure in both the diabetic and normal mouse were consistently in the range of 70–90 mm Hg when anesthetized and 80–100 mm Hg when conscious. Therefore, based on the anatomic and pressure measurements, blood flow in the diabetic mouse is expected to be less than normal. In diabetic humans, reports of both increased (Ditzel, 1975; Butterfield and Holling, 1959) and decreased (Banson and Lacey, 1964; Greeson et al., 1975; Megibow et al., 1953) resting blood flow are available, although a decreased blood flow (Banson and Lacey, 1964; Greeson et al., 1975; Megibow et al., 1953) generally is associated with demonstrable angiopathy. The ability of arterioles to dilate after all vascular control was abolished was depressed for 2A and 4A arterioles of diabetic mice (Table 3), whereas other arterioles exhibited near normal dilatory behavior. The 2A and 4A vessels may simply be near their maximal diameters at rest and, as a consequence, dilate very little in the presence of sodium nitroprusside. Observations in diabetic human subjects of impaired cerebral vascular regulation of blood flow (Bentson et al., 1975) as blood pressure is reduced, depressed dilation of the cutaneous vasculature (Greeson et al., 1975; Megibow et al., 1953), and decreased constrictor responses of retinal arterioles during oxygen inhalation (Hickam and Sieker, 1960) also are indicative of attenuated vascular activity or simply near maximal dilation at rest. Therefore, both the diabetic mouse and human appear to be at a disadvantage to express normal vascular activity, in particular, dilatory responses.

A major finding in the present study is the rapidity of development of arteriolar pathology in the diabetic mouse. All, except the STZ-treated mice allowed to reach an age of 16–18 weeks, were 8–10 weeks old at the time of the study, and the individual diabetic mice, without exception, demonstrated significant arteriolar pathology. As the data in Table 1 indicate, the diabetic mice had been hyperglycemic (> 300 mg%) for 4–6 weeks. Therefore, the duration of an overt diabetic state, as judged by hyperglycemia, was relatively short, and we presume the majority of arteriolar changes occurred in this period. We are confident that the arteriolar changes occurred by the 4th to 5th week of the diabetic state in STZ-treated normal mice because the mice were normal before STZ was administered. We expected the STZ-16 mice, studied at age 16–18 weeks after 12–14 weeks of diabetes, to exhibit more severe vascular changes than in any other mouse model. However, the data recorded for arterioles indicated that the STZ-16 are at a stage of angiopathy that was equivalent approximately to that in other diabetic mice and is indicative of a persistence of juvenile angiopathy to young adult life.

In summary, a decreased number of intermediate and small diameter arterioles, and smaller than normal arteriolar walls, are common characteristics of hyperinsulinemic Ob/Ob and Db/Db diabetic mice and hypoinsulinemic STZ-induced diabetes in mice. These vascular anomalies occur rapidly after the onset of juvenile diabetes and may reflect an impaired growth or maintenance of the microvasculature during juvenile development.

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The Prolonged Pressor Response to Renin in the Nephrectomized Rat

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SUMMARY  Angiotensin I dose-response curves and renin clearances were studied in nephrectomized and paired sham-nephrectomized control rats under pentobarbital anesthesia. Both threshold and slope of the angiotensin I dose-response curves were decreased 22 hours after nephrectomy. In addition, the ratio of renin clearance (determined during renin infusions) in the 22-hour-nephrectomized rat to that in paired 22-hour sham-nephrectomized controls was 0.50 ± 0.03 (mean ± SEM, n = 12 pairs). The finding of reduced renin clearance was confirmed by an indirect assessment of "effective renin clearance" based on a comparison of the blood pressure decline after renin injections with angiotensin I dose-response curves in the same rat. Overall, approximately half of the 50% fall in renin clearance could be accounted for by an immediate effect of removal of the kidney on renin clearance. This role of the kidney in renin clearance was confirmed by the finding of a renal venous-arterial renin response. J Clin Invest 48: 21-29


THE well-recognized prolongation of the pressor response to intravenously administered renin, which develops in the rat over the 24-hour period following nephrectomy, has never been explained satisfactorily. Studies on renin clearance are conflicting (Schaechtelin et al., 1964; Peters-Haefeli, 1971), and even when a decreased clearance has been found after nephrectomy (Bing and Nielsen, 1973), the magnitude of change seems insufficient.
Arteriolar anatomical and functional abnormalities in juvenile mice with genetic or streptozotocin-induced diabetes mellitus.
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