Tissue $P_O_2$ in the Intestinal Muscle Layer of Rats during Chronic Diabetes

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SUMMARY. Chronic diabetes mellitus induced in rats by streptozotocin or of genetic origin in Db/Db mice is associated with a loss of capillaries, arteriolar constriction, and a decrease in resting and maximum blood flow. As a result of these vascular changes, as well as glycolysis of hemoglobin, it is possible that tissue $P_O_2$ is reduced at rest and cannot be substantially increased during dilatation. Tissue $P_O_2$ in the intestinal muscle layer was measured at rest and during maximal dilation. In addition, the spacing between capillaries with active flow and the velocity of red blood cell flow in capillaries were measured at rest and at maximum dilation. These measurements were made in normal and diabetic rats (streptozotocin) at age 26–30 weeks; the diabetic animals had been hyperglycemic (>350 mg/100 ml) for 12–15 weeks. Tissue $P_O_2$ at a distance of about 15 μm from the arterial, mid-point, and venous end of capillaries in normal rats was 24.8 ± 1.1 (SE), 23.1 ± 1.2, and 22.4 ± 0.9 mm Hg, respectively, compared with 25.8 ± 0.9, 24.1 ± 1.2, and 22.4 ± 1.1 mm Hg, respectively, in diabetic rats. The average distance between capillaries was 37.6 ± 2.0 μm in normal rats and 46.8 ± 2.9 μm in diabetic animals; vasodilation did not change the capillary spacing in either group of animals. Capillary red cell velocity in normal rats increased from 0.98 ± 0.11 mm/sec at rest to 2.1 ± 0.4 mm/sec during dilatation. For comparable conditions in diabetic rats, the velocities were 0.41 ± 0.07 and 1.06 ± 0.19 mm/sec. The data presented indicate that the diabetic animals have tissue $P_O_2$ equivalent to those in normal rats, both at rest and during maximum vasodilation. The loss of capillaries and decreased resting and maximum capillary red cell velocity in diabetic rats would decrease the delivery of oxygen, but, apparently, a decrease in oxygen consumption occurred that allowed the intestinal tissue to have a normal $P_O_2$. (Circ Res 52: 677–682, 1983)

THE presence of tissue hypoxia as both a consequence and cause of diabetic microvascular pathology has been proposed by a number of investigators (Korner, 1976; Little, 1976; Ditzel, 1976). In view of the increased oxygen-hemoglobin affinity (Ditzel, 1976; Ditzel et al., 1975) and loss of microvessels (Ditzel and Saglid, 1954; Bohlen and Niggl, 1979), the diabetic man or laboratory animal is faced with a situation favorable to impaired oxygen delivery. However, based on the available data, it is very difficult to predict, even in a general sense, whether and at what stage of vascular pathology the delivery and exchange of oxygen is sufficiently impaired to produce hypoxia. Furthermore, and perhaps of greater importance, the current status of knowledge on diabetic vascular pathology gives relatively little insight as to the relative importance of altered oxygen affinity, loss of capillaries, and impaired vascular control in the generation of impaired vascular exchange.

The purpose of the present study was to determine if the resting and maximum tissue $P_O_2$ in chronically diabetic rats is altered and, if so, by what means. The intestinal muscle layer vasculature was chosen for this study because a prior study (Bohlen and Hankins, 1982) demonstrated that the loss of capillaries and arteriolar pathology in this tissue is qualitatively and quantitatively similar to that in skeletal muscle of diabetic rats and mice (Bohlen and Niggl, 1979a, 1979b, 1980). Furthermore, the anatomical arrangement of capillaries in this tissue is such that the longitudinal gradient of tissue $P_O_2$ from the arterial to venular end of the capillary and associated tissue can be measured readily in the superficial longitudinal and deep circular muscle layer. This is a very important aspect of the study, because it is possible that tissue $P_O_2$ at the arteriolar end of capillaries could be normal but depressed at the venular end of the capillary during diabetes. The tissue $P_O_2$ measurements were correlated to the red cell velocity in capillaries, the red cell transit time in capillaries, and spacing between capillaries to determine whether anomalies in these parameters contributed to alterations in the tissue $P_O_2$ distribution of diabetic animals.

Methods

Male Sprague-Dawley rats (age, 14–15 weeks) were injected intravenously with streptozotocin (40 mg/kg) or the saline vehicle for streptozotocin (STZ). Fifteen saline-injected (to be called normal) and 20 diabetic rats were successfully used for these studies. The normal and diabetic rats were 26–30 weeks old at the time of study; the diabetic rats had been hyperglycemic (>350 mg/100 ml) 12–15 weeks. All animals were anesthetized with Inactin (Byk Gulden Konstanz, West Germany) (100 mg/kg, ip). Supple-
Fluid replacement (Normasol-R, Abbott Labs.) equal to 0.5 ml per hour was given to assist in the maintenance of a constant arterial blood pressure. A femoral artery was cannulated to measure arterial pressure, and the trachea was cannulated to ensure an open airway.

The small intestine was prepared for microscopic observation by means of a previously described technique (Bohlen, 1980). The intestine and body were warmed to maintain a temperature of 37-38°C. The intestine was bathed in a flowing (3 ml/min) physiological solution with a pH of 7.35 ± 0.05, and Po2 and Pco2 of 40-45 mm Hg, as measured in the fluid over the tissue. The environment created for the bowel closely mimicked conditions of pH and gas tensions in the abdominal cavity (Bohlen, 1980).

After surgery, the animal and tissue were allowed to stabilize for 30-40 minutes. The measurement period lasted 3-4 hours after the stabilization period. Data collection was terminated when either the mean arterial pressure decreased more than 15 mm Hg compared with that after the stabilization period, or white blood cells stuck to the inner walls of venules. This latter factor was not of particular use in diabetic animals because white cells were lodged in venules at the beginning of experiments, and the severity of this problem slowly increased during the experiment. In general, technical rather than biological problems limited the duration of experiments on normal and diabetic animals.

Red cell velocity was measured with a dual-slit cross-correlation technique based on the method developed by Wayland and Johnson (1967) and manufactured by Instrumentation for Physiology and Medicine, Inc. The velocity system was calibrated by measurement of the velocity of whole blood (hematocrit 35-45%) suspended on a rotating clear plastic wheel whose radius and rotation frequency were known.

Tissue Po2 was measured with recessed-tip (15-20 μm), gold-plated electrodes similar in form and function to those developed by Whalen et al. (1973). The electrode tip was sharpened to a diameter of 1.5-2.5 μm to facilitate penetration of tissue. The electrodes were calibrated and tested for a linear current-Po2 relationship in a precision tonometer (Proctor and Bohlen, 1979). The 0 mm Hg tonometer Po2 was compared with the electrode current measurement in freshly prepared yeast cultures. The currents measured in both situations were nearly equal, although the current was usually slightly lower in the yeast than tonometer. If the electrode current was less than 10^{-4} amps (Po2 = 140 mm Hg), the current-Po2 relationship linear (±5%), and a current-voltage plateau of 0.15 V or more was present, the electrode was used. During each experiment, the electrode calibration was confirmed after every other measurement.

The protocol for each experiment began by random selection of capillaries in either the outer longitudinal or inner circular muscle layer. Thereafter, use of capillaries in the two muscle layers was alternated. The length of each capillary and distance between adjacent capillaries then was measured. Prior to electrode penetration, the red cell velocity was measured. The electrode then was placed about 15 μm from the capillary at the arterial or venous end. The electrode current was less than 10^{-4} amps (Po2 = 140 mm Hg), the current-Po2 relationship linear (±5%), and a current-voltage plateau of 0.15 V or more was present, the electrode was used. During each experiment, the electrode calibration was confirmed after every other measurement.

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In some experiments, the tissue Po2 in villi was measured by turning the preparation over to expose villi. The location of tissue Po2 measurement in each villus was always near the mid-point of the villus height.

The hematocrit, plasma insulin concentration, fraction of the total hemoglobin in the form of glycolysed hemoglobin (HbAlc), and plasma glucose concentration were measured in conscious, fasted (12-hour) animals. The blood needed for these measurements was obtained from the tail vein or by cutting the tip of the tail. The plasma glucose was determined with a Beckman model 2 glucose analyzer. The glycolysed hemoglobin fraction and insulin concentration measurements were made using Heding's (1972) technique and the Isolab, Inc., test kit, respectively.

Statistical analysis was made by means of a t-test for equal or unequal numbers of observations (Sokol et al., 1969). A P value of 0.05 or less was accepted as an index of significant difference between the means. All data are reported as the mean ± SEM.

Results

Animal Characteristics

The physical and chemical status of each animal studied is summarized in Table 1. As a result of insulin depletion, the diabetic rats were severely emaciated and hyperglycemic, as demonstrated by the data in Table 1. The hematocrit of diabetic animals was slightly but significantly (P < 0.05) decreased. The percentage of the total hemoglobin in the form of glycolysed hemoglobin (%HbAlc) was 7.5 ± 0.3% of the total, or an increase of about 4% of total hemoglobin above normal. During the anesthetized state, the mean arterial blood pressures in normal and diabetic animals were not different (P > 0.05), so long as the diabetic animals received replacement fluid. Acute volume expansion (1-2 ml/100 g) did not cause more than a temporary (30-minute) increase in arterial pressure in either normal or diabetic animals. However, continuous volume replacement was essential to maintain a constant blood pressure in diabetic rats due to their exaggerated diuresis.

Capillary Flow Properties, Length, and Spacing

The upper panel of Figure 1 presents the mean capillary red cell velocity for resting and passive conditions. The red cell velocity in diabetics was about half of normal for both resting and passive conditions. For the passive state, caused by maximum dilation with topically applied adenosine (10^{-4} M), the red cell velocity of flow in diabetics did increase enough to be equal to that in normal animals during resting conditions. The passive state caused dilation of only the section of bowel under study, and did not alter the mean arterial blood pressure.
TABLE 1
Physical and Metabolic Characteristics of Normal and Diabetic Rats

<table>
<thead>
<tr>
<th></th>
<th>Normal (n = 15)</th>
<th>Diabetic (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (wk)</td>
<td>26-29</td>
<td>26-29</td>
</tr>
<tr>
<td>Mean arterial</td>
<td>114 ± 4</td>
<td>219 ± 6*</td>
</tr>
<tr>
<td>pressure (mm Hg)</td>
<td>401 ± 5</td>
<td>12-15</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>112.8 ± 2.2</td>
<td>639 ± 11*</td>
</tr>
<tr>
<td>Duration of</td>
<td>26.6 ± 2.7</td>
<td>7.2 ± 1.5*</td>
</tr>
<tr>
<td>hyperglycemia (wk)</td>
<td></td>
<td>7.5 ± 0.3*</td>
</tr>
<tr>
<td>Plasma glucose (mg/100 ml)</td>
<td>3.5 ± 0.1</td>
<td>49.5 ± 0.7*</td>
</tr>
<tr>
<td>Plasma insulin (µU/ml)</td>
<td>52.8 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Glycolysated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hematoglobin (% of total)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematocrit</td>
<td>3.5 ± 0.1</td>
<td>49.5 ± 0.7*</td>
</tr>
</tbody>
</table>

* Asterisks denote a significant difference (P < 0.05) between normal and diabetic animals.

The transit time of red cells in capillaries, shown in the lower panel of Figure 1, was calculated from the length of the capillary divided by the red cell velocity in that capillary. The average length of capillaries was 208.5 ± 15.5 µm in normal animals and is not different (P > 0.05) from 226.5 ± 16.4 µm in diabetic rats. In addition, capillary lengths for longitudinal and circular muscle layer vessels were statistically equal (P > 0.05). The transit time or, perhaps more relevant to transcapillary exchange, residence time of a red cell in a capillary was more than twice as long as normal in diabetics for both rest and passive conditions. The transit-residence time was increased in diabetics due exclusively to subnormal red cell velocity in a normal length capillary.

The average distance between adjacent capillaries with active flow along an arteriole was 37.6 ± 2 µm (n = 70 arterioles) in normal rats and 46.8 ± 2.9 µm (n = 86 arterioles) in diabetic rats. The spacing between capillaries did not change during passive conditions in either normal or diabetic rats. This indicated that nearly all capillaries were in use under resting conditions in normal and diabetic rats. The distributions of capillary separation distances for diabetic and normal rats were very similar to those reported (Bohlen and Hankins, 1982) for the intestine of diabetic rats after 4-5 weeks of hyperglycemia and normal rats, respectively, at an age 6-9 weeks younger at the time of study than those used in the present investigations.

The flow per actual mass of tissue was not available due to technical limitations of separating the muscle layer from the remainder of the bowel. However, the relative flow per unit volume of tissue can be calculated from the number of capillaries per unit volume, which is about 75-80% of normal based on capillary separation distances, and average flow per capillary. The resting flow in diabetic rats was 40-60% of normal and the maximum flow was 40-60% of the maximum flow in normal rats. Normal rats increased their maximum flow to 200-220% of resting flow during maximum dilation. As shown in Table 1, hematocrits in normal and diabetic rats were very similar; therefore, red cell flux (velocity * HCT) would correspond to the same percentages listed for red cell flow.

Tissue PO2

The tissue PO2 measurements to be presented were made when the tissue was bathed constantly by a physiological solution with a PO2 of 40-45 mm Hg. This PO2 represented the typical PO2 of fluid aspirated from the abdominal cavity of conscious but sedated
rats in a prior study (Bohlen, 1980). Therefore, the suffusion P_{O2} of 40–45 mm Hg closely duplicated the oxygen environment of the intestinal muscle layer during intact conditions. There is a possibility that the suffusion P_{O2}, even though at a physiological pressure appropriate for the intestinal muscle tissue, influenced the tissue P_{O2}. As stated in Methods, tissue P_{O2} and capillary characteristics were recorded in both the superficial longitudinal and deeper circular muscle layers of normal and diabetic rats. The tissue P_{O2} and capillary velocity, length, and spacing characteristics of the two layers in a given animal type were not different and have been averaged together. Had the suffusion solution overtly influenced the outer tissue layer, a higher tissue P_{O2} would have probably existed in the outer compared with the inner muscle layer. This did not occur in normal or diabetic rats. In addition, the tissue P_{O2} just within the surface of the tissue was not systematically higher or lower than at deeper points in the superficial muscle layer, over a depth of 30–50 μm. Had the suffusion P_{O2} been a factor in either normal or diabetic rats, a gradual decrease in tissue P_{O2} would have occurred as the electrode tip was advanced into the tissue. Even if the suffusion solution did contribute oxygen to the tissue, the amount of oxygen was probably very small. In support of this statement, if the blood flow to the intestine of normal or diabetic rats was stopped by arterial occlusion or killing the animal, the recorded tissue P_{O2} was not detectably different from the 0–3 mm Hg P_{O2} with the pipette tip just within the tissue surface. The use of a suffusion solution in these experiments was absolutely necessary to allow pipette penetration, prevent tissue dehydration, and protect the tissue from atmospheric oxygen. The P_{O2} of the solution used duplicated the natural environmental P_{O2} of the abdominal cavity, and, based on the tests just described, did not appear to have a different influence on the recorded P_{O2} in normal and diabetic rats.

The average tissue P_{O2} measurements at the arterial, mid-point, and venous end of the tissue around a capillary are shown in Figure 2. Despite differences in numbers of capillaries and capillary flow velocities, the diabetic animals had tissue P_{O2} equal (P > 0.05) to those of normal animals. The gradient of tissue P_{O2} (P_{O2}/length) from the arterial to venous end of the tissue around a capillary was 1.5–1.75 mm Hg/100 μm in normal and diabetic animals. The radial variation of P_{O2} at various distances from a given capillary was so small that—from the capillary wall to half-way between adjacent capillaries—it usually was not possible to detect a P_{O2} gradient. This occurred in part because the electrodes used were reliably capable of detecting a change in P_{O2} in tissue of no greater than 1–2 mm Hg due to both limitations of the electrode, placement of electrodes, and slow movements of the muscle tissue. In addition, capillaries adjacent to the point of study probably contributed oxygen, which further impaired the precise ability to detect both radial and longitudinal P_{O2} gradients. This auxiliary addition of oxygen became acutely obvious when it was found that if the oxygen microelectrode was first placed directly on the capillary wall and then the pipette gently occluded the capillary, the measured P_{O2} decreased only 3–7 mm Hg. However, it is important to prevent a mechanical compression of the capillaries during pipette penetration, because partial obstruction of capillary flow invariably reduced the recorded tissue P_{O2} compared with normal flow conditions.

Villus P_{O2} at mid-height of the villus was 19.8 ± 0.8 mm Hg (60 observations, eight animals) in diabetic rats compared with 21.3 ± 1.0 mm Hg (40 observations, six animals) and was not different (P > 0.05). Villi of diabetic and normal animals are both tongue-shaped and 500–700 μm tall. However, the core tissue in villi of diabetic animals was very sparse, so that the villi were thin compared with those of equal-aged normal animals. What, if any, effect the different thicknesses of villi might have on tissue P_{O2} is not known. The number of villi per 8 mm² in normal rats was 93.8 ± 2.1 compared with 64.3 ± 2.0 in diabetic animals.

The P_{O2} of arterial and venous blood, as well as in the vessel walls of arterioles and venules, was measured to ensure that the blood traveling to the microvasculature was at equal oxygen tensions in normal and diabetic animals. Due to oxygen loss through the
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Discussion

The results presented in Figure 2 indicate that—in both normal and diabetic rats—there was a gradual but significant (P < 0.05) decrease in tissue PO₂ of 2–3 mm Hg from the arterial to venous end of the capillary bed. The arterial-to-venous gradient of PO₂ referred to the capillary length was 1.5–1.75 mm Hg/100 μm in both normal and diabetic animals. These data, in conjunction with the observation of a very small radial gradient of PO₂ around the capillary, indicate that the rat intestinal muscle tissue has a very homogeneous tissue PO₂ distribution. Based on Krogh’s (1918) mathematical model of the radial and longitudinal distribution of tissue PO₂ about a single capillary, one would expect a greater variability of tissue PO₂ along the capillary length than was measured. However, if the diffusion fields of capillaries in the same tissue plane, as well as adjacent tissue planes, are extensively overlapped, Krogh’s (1918) model would also indicate a minor radial and longitudinal gradient of tissue PO₂ as was found in this study. As stated in Results, occlusion of a single capillary in the outer longitudinal or inner circular muscle layer vasculature caused a 3–7 mm Hg decrease in tissue PO₂. Therefore, the tissue PO₂ at any given location was a function of both the local capillary flow and diffusion of oxygen into the area from adjacent capillaries. This situation probably occurred in the intestinal muscle vasculature because virtually all capillaries are perfused during resting conditions and an overlap of capillary diffusion fields occurred. Whether a relatively homogeneous tissue PO₂ distribution exists along the capillary length in other tissues has not been documented, as was recently pointed out by Honig and Odoroff (1981).

The absence of a significant difference in resting PO₂ in intestinal villi and at comparable locations along the capillary length in the muscle layer of normal and diabetic rats, as shown in Figure 2, was unexpected. The diabetic vasculature has two major characteristics which should have decreased the tissue PO₂. First, the average distance between muscle layer capillaries was 37.6 ± 2 μm in normal rats and 46.8 ± 2.9 μm in diabetic animals. Second, based on the red cell velocity and spacing of capillaries, the resting and maximum blood flow in diabetic rats was of the order of 40–60% of that in normal animals, both at rest and during maximum dilation. A third factor which had the potential to impair oxygen exchange was a 4% increase in the fraction of hemoglobin as glycolysated hemoglobin (Table 1) (Ditzel and Saglid, 1954; Ditzel et al., 1975; Ditzel, 1976). Yet, with these factors present, the resting and maximum tissue PO₂ measurements in the diabetic animals were equivalent to those in normal rats. Possibly, the best tentative explanation of equal tissue PO₂ in normal and diabetic animals was a decreased oxygen usage in diabetic animals. Specific information on visceral smooth muscle oxygen use during chronic, severe diabetics is not available. However, both skeletal muscle (Chen and Ianuzzo, 1982) and vascular smooth muscle (Wolinsky et al., 1978) in streptozotocin-treated rats are known to have reduced enzymatic activity related to aerobic metabolism unless treated with insulin. Therefore, it is possible that the metabolic impairments of the severe diabetic state constrain oxygen usage such that resting tissue PO₂ can remain about normal despite the microvascular pathology. I have considered the possibility that the suffusion solution was a source of oxygen for the tissue and caused approximately equal tissue PO₂ in normal and diabetic animals. However, the suffusion solution PO₂ used closely mimicked intra-abdominal fluid PO₂ such that if this PO₂ had an effect on tissue, approximately equal effects should have occurred in intact situations as well as during experimental conditions. Two additional observations indicated a minor effect of the suffusion solution PO₂ on tissue PO₂. Advancing the oxygen electrode from the tissue surface to 30–50 μm into the tissue had little or no measurable effect on the recorded PO₂ in normal or diabetic rats. Second, when blood flow was stopped, the tissue PO₂ recorded near the tissue surface was not detectably different from a 0–3 mm Hg PO₂ in either animal group. In designing this study, I considered the possibility that the PO₂ of blood as it reached the smallest arterioles was different in normal and diabetic rats. However, as described in Results, the intravascular PO₂ values at comparable locations along the arteriolar vasculature were equivalent in normal and diabetic animals. These observations also have a bearing on the role of oxygen in arteriolar vessel wall damage during diabetics. The similarity in luminal and external vessel wall PO₂ in normal and diabetic animals...
was so great that it was unlikely that the arteriolar wall was hypoxic in diabetic animals. Therefore, if tissue oxygenation is a factor in arteriolar wall pathology, a deleterious effect would have to be caused by some indirect mechanism, or the approximately normal vessel wall PO2 is inadequate for the needs of the arterioles in diabetic animals.

The observations made on capillary spacing along arterioles in normal and diabetic rats offered a useful insight into the development of capillary pathology in the diabetic rat. In a previous study (Bohlen and Hankins, 1982) using rats from the same colony as in the present study and made hyperglycemic at the same age and severity, the average intestinal muscle layer capillary spacing was 44.6 ± 1.1 μm after 4–5 weeks of hyperglycemia compared with 46.8 ± 2.9 μm in the present study after 12–15 weeks of hyperglycemia. The capillary spacing in normal animals of the previous study was 33.8 ± 1.4 μm and 37.6 ± 2 μm in the current study. Apparently, the loss of capillaries in diabetic animals occurred very early in the disease process and did not appreciably change in severity thereafter. In addition, the lengths of capillaries in the present normal and diabetic animals were not statistically different (P > 0.05). Therefore, the increased spacing between capillaries may have represented a simple loss of capillaries rather than a pathological process which affected both the spacing and length of capillaries. In previous studies of the skeletal muscle vasculature during genetically induced diabetes in Db/Db mice (Bohlen and Niggl, 1979a, 1979b), the loss of capillaries also occurred in the initial stages of diabetes, and capillary spacing remained stable thereafter. The combined data from the studies using streptozotocin (Bohlen and Hankins, 1982) and genetic models of diabetes (Bohlen and Niggl, 1979a, 1979b) therefore indicated that the loss of capillaries was related to the early stages of the diabetic process, and was not a consequence of the injection of streptozotocin.

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INDEX TERMS: Diabetes • Microcirculation • Tissue PO2

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