Tissue $\text{PO}_2$ 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 $\text{PO}_2$ is reduced at rest and cannot be substantially increased during dilation. Tissue $\text{PO}_2$ 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 $\text{PO}_2$ at a distance of about 15 $\upmu$m 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 maximum mid-capillary tissue $\text{PO}_2$ during dilation was 27.7 ± 1.3 mm Hg in normal rats and 29.7 ± 1.5 mm Hg in diabetic rats. The average distance between capillaries was 37.6 ± 2.0 $\upmu$m in normal rats and 46.8 ± 2.9 $\upmu$m 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 $\text{PO}_2$ 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 $\text{PO}_2$.

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mental doses of anesthetic (10 mg/kg, ip) were given at approximately 2-hour intervals.

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 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 10⁻¹⁰ 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 15 μm out from the mid-point of the capillary. The red cell velocity was re-measured to ensure no change had occurred after each pipette penetration. The tissue then was flooded with gassed suffusion solution (5% O2, 5% CO2) which had the same gas tensions as the routine fluid but contained 10⁻⁴ M adenosine to cause maximal dilation. The adenosine suffusion was continued until the tissue PO2 increased to a new equilibrium, which usually occurred in 2-3 minutes. The average red cell velocity during maximal dilation was measured after the tissue PO2 reached the new, higher steady state.

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 glycolysated 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 glycolysated 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 glycolysated 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

<table>
<thead>
<tr>
<th></th>
<th>Normal (n = 15)</th>
<th>Diabetic (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean arterial pressure</strong></td>
<td>114 ± 4 mm Hg</td>
<td>219 ± 6*</td>
</tr>
<tr>
<td><strong>Weight (g)</strong></td>
<td>401 ± 5</td>
<td>12-15</td>
</tr>
<tr>
<td><strong>Duration of hyperglycemia (wk)</strong></td>
<td>112.8 ± 2.2</td>
<td>639 ± 11*</td>
</tr>
<tr>
<td><strong>Plasma glucose (mg/100 ml)</strong></td>
<td>26.6 ± 2.7</td>
<td>7.2 ± 1.5*</td>
</tr>
<tr>
<td><strong>Plasma insulin (IU/ml)</strong></td>
<td>3.5 ± 0.1</td>
<td>7.5 ± 0.3*</td>
</tr>
<tr>
<td><strong>Glycylated hemoglobin (% of total)</strong></td>
<td>52.8 ± 0.7</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 by 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 \( P_{O_2} \) 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 \( P_{O_2} \), even though at a physiological
pressure appropriate for the intestinal muscle tissue,
influenced the tissue \( P_{O_2} \). As stated in Methods, tissue
\( P_{O_2} \) and capillary characteristics were recorded in
both the superficial longitudinal and deeper circular
muscle layers of normal and diabetic rats. The tissue
\( P_{O_2} \) and capillary velocity, length, and spacing char-
acteristics 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 \( P_{O_2} \) 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 \( P_{O_2} \) 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 \( \mu m \). Had the suffusion \( P_{O_2} \) been
a factor in either normal or diabetic rats, a gradual
decrease in tissue \( P_{O_2} \) 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 \( P_{O_2} \) was not detectably different from the 0–3
mm Hg \( P_{O_2} \) 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 \( P_{O_2} \) of
the solution used duplicated the natural environmental
\( P_{O_2} \) of the abdominal cavity, and, based on the tests
just described, did not appear to have a different
influence on the recorded \( P_{O_2} \) in normal and diabetic
rats.

The average tissue \( P_{O_2} \) measurements at the arte-
rial, 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 \( P_{O_2} \) equal (\( P > 0.05 \))
to those of normal animals. The gradient of tissue \( P_{O_2} \)
(\( P_{O_2}/\)length) from the arterial to venous end of
the tissue around a capillary was 1.5–1.75 mm Hg/100
\( \mu m \) in normal and diabetic animals. The radial vari-
ation of \( P_{O_2} \) 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 pos-
sible to detect a \( P_{O_2} \) gradient. This occurred in part
because the electrodes used were reliably capable of
detecting a change in \( P_{O_2} \) 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 \( P_{O_2} \) 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
\( P_{O_2} \) decreased only 3–7 mm Hg. However, it is im-
portant to prevent a mechanical compression of the
capillaries during pipette penetration, because partial
obstruction of capillary flow invariably reduced the
recorded tissue \( P_{O_2} \) compared with normal flow con-
ditions.

Villus \( P_{O_2} \) 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 observa-
tions, six rats) and was not different (\( P > 0.05 \)). Villi
of diabetic and normal animals are both tongue-
shaped and 500–700 \( \mu m \) 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 \( P_{O_2} \) is not
known. The number of villi per 8 \( \mu m^2 \) in normal rats
was 93.6 ± 2.1 compared with 64.3 ± 2.0 in diabetic
animals.

The \( P_{O_2} \) of arterial and venous blood, as well as in
the vessel walls of arterioles and venules, was meas-
ured to ensure that the blood traveling to the micro-
vasculature was at equal oxygen tensions in normal
and diabetic animals. Due to oxygen loss through the
arteriolar wall into the tissue, the arterial blood Po2 in the largest arterioles was 56.5 ± 2.2 mm Hg in normal animals and 53.3 ± 2.8 mm Hg in diabetic rats. These Po2 measurements were made in the largest arterioles approximately 3 mm from the point at which they entered the tissue. Vasodilation would increase the Po2 measured in larger arterioles by 20–25 mm Hg. The Po2 just outside the vessel wall was typically less than 5 mm Hg lower than that in the blood, as has also been observed by Duling (1972) in hamster arterioles. The Po2 measurements in the wall of subsequent branches of the arterial system were in the range of 30–45 mm Hg in normal and diabetic rats, with the lower values associated with arterioles which perfused the arterioles of the muscle layer. The Po2 on the walls of the smallest venules and in the blood of these vessels were 20–25 mm Hg, and usually not distinguishable from tissue Po2 in the region. However, venules that lay beside arterioles apparently pick up oxygen lost from the arteriolar wall, because venous blood and vessel wall Po2 gradually increased in successively larger venules. In the largest venules, venous blood Po2 values of 33.7 ± 1.8 mm Hg and 34.4 ± 1.6 mm Hg were recorded in normal and diabetic animals, and these values increased relatively little (3–7 mm Hg) during maximum dilation.

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 referenced 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 glycosylated 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 P02 is inadequate for the needs of the arterioles in diabetic animals.

The observations made on capillary spacing among 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 \pm 1.1 \mu m after 4–5 weeks of hyperglycemia compared with 46.8 \pm 2.9 \mu m in the present study after 12–15 weeks of hyperglycemia. The capillary spacing in normal animals of the previous study was 33.8 \pm 1.4 \mu m and 37.6 \pm 2 \mu m 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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