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Red Cell Velocities in the Mesenteric Microvessels of Streptozotocin-Diabetic Rats

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RED CELL VELOCITIES IN THE MESENTERIC MICROVESSELS OF STREPTOZOTOCIN-DIABETIC RATS

by
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BIOGRAPHY

Karl M. Nelson is the son of Harold E. and Anna-Margaret (Hansen) Nelson. He was born September 15, 1950, in Albert Lea, Minnesota.

He attended Lakes High School, Tacoma, Washington, where he graduated in 1968.

In September, 1968, he entered Pacific Lutheran University in Tacoma, Washington where he received the Bachelor of Arts degree in Biology in 1972. He spent one and a half years at the University of Washington, Seattle, Washington studying zoology before beginning his graduate education.

He began his graduate studies in the Department of Physiology at the Loyola University Stritch School of Medicine in July, 1974. He worked under the direction of Dr. George P. Pollock.

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CHAPTER I

INTRODUCTION

No disease so uniformly affects the microvasculature as diabetes mellitus. The chronic degenerative disease of the microvascular system which occurs in diabetes mellitus was first recognized over a century ago [reference cited in 71]. Elucidation of the nature of diabetic microangiopathy was extremely slow until the discovery of insulin in 1922. The advent of insulin therapy brought a longer life after the onset of measurable biochemical abnormalities. Consequently, more extensive vascular disease has developed, so that today 85% of the people with diabetes mellitus die from the cardiovascular-renal complications of diabetic microangiopathy rather than from ketoacidosis, insulin shock and other related conditions.

The microvascular manifestations of diabetes mellitus represent a far more serious aspect of this disease than do the carbohydrate derangements. "Tightly controlled" diabetics develop vascular problems as readily as insulin-independent diabetics' [91]. Yet little is known about the basic pathogenesis of diabetic microangiopathy. With recognition of the importance of diabetic microangiopathy, there is an appreciation of the need to evaluate quantitatively how diabetes mellitus alters microcirculatory phenomena.

The purpose of this study was to evaluate quantitatively the microcirculation in an animal model of diabetes mellitus. Specifically, the red cell velocity in mesenteric microvessels, i.e., arterioles, capillaries and venules, were measured in non-diabetic and streptozotocin-diabetic rats.

CHAPTER II

LITERATURE REVIEW

A. PHYSICAL AND CHEMICAL ALTERATIONS OF THE CAPILLARY BASEMENT MEMBRANE IN DIABETES MELLITUS

1. Physical Changes in the Diabetic Capillary Basement Membrane

The term "diabetic microangiopathy" denotes the chronic, degenerative vascular disease of the smallest blood vessels throughout the body in diabetes mellitus [24, 30, 54, 79]. Using histochemical techniques, light microscopic examination of various tissues revealed the presence of increased amounts of periodic acid-Schiff (PAS) positive material in the vessel walls of arterioles, capillaries and venules [30, 39, 65, 79, 107]. Basement membranes are extra-cellular structures that react intensely with periodic acid-Schiff stain. On the basis of light microscopic studies, Friedenwald [34, 35] suggested that a specific thickening of the capillary basement membrane contributed to the microvascular complications of diabetes mellitus. Electron microscopy provided the technology with which the validity of this suggestion could be examined.

The basement membrane located under the endothelium of blood vessels appears as an electron dense feltwork of fine fibrils when seen on electron micrographs [58]. It is usually only a few hundred angstroms in width [79]. When the same localization is demonstrated with PAS staining techniques and viewed with a light microscope, it appears as a homogeneous structure and measures about 1 to 2 microns in width [79]. The basement membrane seen on light microscopy and electron microscopy are two different things.

The first electron microscopists simply borrowed a term in common usage among light microscopists.

Electron microscopy was used extensively by Siperstein et. al. [92] and Williamson and his group [53, 123] as a tool for quantitatively assessing basement membrane thickening in diabetics. Their anatomical studies demonstrated that a segmental thickening of the capillary basement membrane is the morphological feature which best characterizes diabetic microangiopathy [53, 124]. Arterioles and venules may be similarly affected [30, 122]. Other investigators reported similar observations in such diverse tissues as skin [1, 65, 79], muscle [53, 79, 92, 115, 116], retina [8, 79] and kidney [7, 32, 78, 79]. This suggests that thickening of the capillary basement membrane may be a generalized phenomenon involving small blood vessels throughout the body [30, 54, 71, 96, 107, 124].

While thickening of the capillary basement membrane is generally accepted as one of the first manifestations of diabetic microangiopathy, the electron microscopic observations have become disputed in two areas. These are: (1) the time of the occurence of thickening in relation to the onset of carbohydrate derangements and (2) whether basement membrane thickening progresses with duration of diabetes. Siperstein proposed that basement membrane thickening occurs before the onset of diabetes and may be the cause and not the consequence of diabetes. Østerby-Hansen [78] found that diabetic microangiopathy and thickening of the basement membrane do not occur in the glomeruli of young diabetics of recent onset. This indicates that early changes in prediabetes is unlikely. Williamson [53] suggests that the basement membrane width of muscle capillaries is normal in most individuals prior to the onset of carbohydrate intolerance.

According to Pometta et. al. [82] there is no significant increase in the basement membrane width of muscle capillaries in potential diabetics or diabetics of recent onset as compared to non-diabetic control subjects.

Siperstein [92] has reported that no relationship exists between the duration and severity of diabetes and the width of the capillary basement membrane. If generalized basement membrane thickening precedes carbohydrate intolerance, then basement membrane thickening should be found in all diabetics. That Williamson [125] found basement membrane thickening in less than one third of insulin-dependent diabetics who have had their disease less than four years, coupled with a highly significant increase in the incidence of basement membrane thickening with increased duration of carbohydrate intolerance supports the concept that basement membrane thickening is a consequence of the carbohydrate intolerance associated with diabetes.

The disparate results responsible for the controversy regarding the incidence and significance of capillary basement membrane thickening in diabetes mellitus can be reconciled on the basis of the following: (1) methods of fixation and measurement technique [52, 94, 120, 123, 125]; (2) artifacts related to criteria for selection of subjects [52, 120, 125]; (3) sample size [52, 120]; and (4) the use of age and sex adjusted normal confidence limits in assessing basement membrane thickening.

2. Chemical Changes in the Diabetic Capillary Basement Membrane

The periodic acid-Schiff staining technique is very discriminating in detecting glycoproteins in tissue sections [81, 99, 107]. Basement membranes react intensely with PAS stain. This indicates their glycoprotein nature [101]. Compositional analyses of basement membranes have supported the histochemical evidence concerning their glycoprotein nature [101, 105].

On the basis of histochemical evidence it was postulated that diabetic microangiopathy resulted from a disturbance in the metabolism of the carbohydrate containing proteins [35]. Studies of the glomerular basement membrane in human diabetes indicated that the total amount of membrane present is increased and furthermore that a distinct chemical alteration occurred [5, 6, 57]. The chemical alteration in the basement membrane was found to specifically involve an increased amount of hydroxylysine and a similar elevation in the number of hydroxylysine attached glucosylgalactose disaccharide units.

Spiro proposed a sophisticated hypothesis to explain the alteration in chemical composition [104, 105]. He found that the level of kidney glucosyltransferase, an enzyme involved in the synthesis of the hydroxylysine-linked disaccharide units of the glomerular basement membrane, was significantly elevated in alloxan diabetic rats when compared to age matched controls [102]. He suggested that the elevation of carbohydrate content in the diabetic glomerular basement membrane is not simply due to an increased attachment of glucosylgalactose disaccharide units to unsubstituted hydroxylysine residues. In the normal glomerular basement membrane, all sterically available hydroxylysine residues are glycosylated [103]. Spiro [104, 105] postulated that the increase in the carbohydrate content of the diabetic glomerular basement membrane is due to a preferential production of subunits rich in hydroxylysine, rather than to an increase in hydroxylation of lysine residues already present. These newly formed hydroxylysine residues are subsequently glycosylated.

The validity of this hypothesis was challenged by Kefalides [51] and Westberg and Michael [120]. These investigators have performed

compositional analyses on the human diabetic glomerular basement membrane with variable results. Westberg and Michael [120] found that the most distinct difference in the amino acid composition between glomerular basement membranes from normal and diabetic kidneys was the decrease in the half cystine content of the diabetic kidneys. Contrary to the findings of Beisswenger and Spiro [6], they found no increase in the amounts of hydroxylysine or in the number of glucosylgalactose disaccharides linked to this amino acid. Kefalides [51] found no difference in the half cystine content of normal and diabetic glomerular basement membranes. His data demonstrated that the amino acid and carbohydrate compositions of the diabetic glomerular basement membrane are very similar to those of the normal glomerular basement membrane.

Spiro [105] has pointed out a number of pitfalls to be avoided in compositional analyses of glomerular basement membranes. The kidneys to be analyzed for chemical changes must be limited to those showing microscopic evidence of glomerular pathology. Care must be taken that pathological glomeruli are isolated and that the preparation is not contaminated with cell membranes. The pathological changes in the kidneys used by Westberg and Michael [120] and Beisswenger and Spiro [5, 6] were compatible with diabetic kidney disease. Differences in the purity of the glomerular basement membrane preparations could possibly explain the discrepancy between the results of Westberg and Michael [120] and those reported by Beisswenger and Spiro [5, 6]. Kefalides [51] does not give any information about the presence or absence of glomerular pathology in the kidneys he used in performing his compositional analyses. In addition, his preparations were probably contaminated with cell membranes.

3. Summary of Basement Membrane Alterations in Diabetes Mellitus

Studies of microvessels in diabetes in the last fifteen years have concentrated heavily on the anatomic and biochemical abnormalities of the capillary basement membrane [5, 6, 52, 53, 57, 71, 78, 79, 82, 92, 94, 104, 105, 115, 116, 123, 124, 125]. The anatomical studies demonstrated that a segmental thickening of the capillary basement membrane characteristically occurs in diabetics. The biochemical studies suggest that the chemical structure of the basement membrane may be altered. Controversy has developed about both of these changes. This has had the unplanned effect of diminishing attention to the overall goal, i.e., elucidation of the relationship between the insulin-deficient state and the development of the microvascular concomitants of diabetes mellitus. It remains to be established how these anatomic and biochemical changes influence the physiologic role of the capillary basement membrane as a supportive structure and selective filter.

B. PERMEABILITY OF SMALL VESSELS IN DIABETES MELLITUS

The periendothelial deposition of PAS(+) material and the pronounced thickening of the capillary basement membrane in diabetic microangiopathy has implications for transcapillary transport. Westberg and Michael [120] and Spiro [5, 100, 103, 104] have suggested that the changes they find in the chemical composition of the glomerular basement membrane could alter the porosity of the membrane. This would be due to the compositional changes affecting cross-links between peptide chains or by sterically interfering with their packing.

1. Permeability of Skeletal Muscle Capillaries

Various investigators [2, 80, 110, 111, 112, 113, 114] studied the transfer of electrolytes and large molecules across the capillary membrane between the vascular compartment and the interstitial compartment of skeletal muscle in long-term (>10 years) and short-term (<5 years) juvenile diabetics. Their findings suggest that the skeletal muscle capillaries of long-term diabetics are more permeable. This increase in permeability was attributed to an increase in the diameter of the intracellular cleft from 40 to 50 angstroms [114]. It was not attributed to an increase in the porosity of the basement membrane or to an increase in the area of the exchange surface [2]. The thickened capillary basement membrane is not the rate limiting factor in the transcapillary exchange process in skeletal muscle.

2. Permeability of Glomerular Capillaries

The basement membrane surrounding glomerular capillaries is regarded as the main filter preventing passage of large molecules in the kidney. A more direct application of the theories of molecular sieving is possible in the glomerulus. The filtration rate can be determined with a high degree of accuracy and complete collection of the filtered macromolecules is possible if insignificant tubular reabsorption takes place. The suggestion that changes in the chemical composition of the glomerular basement membrane alters the porosity of the membrane [5, 6, 100, 103, 104, 105, 120] ought to be studied in the kidney rather than in skeletal muscle.

C.E. Mogensen [72, 73, 74] studied the glomerular filtration rate (GFR) and glomerular permeability to macromolecules in newly diagnosed, untreated juvenile diabetics and in short-term and long-term juvenile

diabetics. He found that the GFR was increased 41% in the newly diagnosed, untreated diabetics when compared with age matched controls [74]. The renal plasma flows obtained by PAH and ^{131}I-hippuran clearances were not different from normal values. If a more porous basement membrane was responsible for the increase in GFR, then the clearance of larger molecular weight dextrans should increase. Mogensen [72, 73] found no evidence to indicate this. Mogensen [74] also found that the GFR decreased as the duration of diabetes increased. This is not consistent with the suggestion of Westberg and Michael [120] or Beisswenger and Spiro [5, 6] that changes in the chemical composition of the glomerular basement membrane alters its porosity.

3. Summary of Permeability Changes in Diabetic Capillaries

The permeability studies of skeletal muscle and glomerular capillary basement membranes strongly suggest that the thickened basement membranes of diabetics do not hinder transcapillary exchange processes. The glomerular permeability to macromolecules does not indicate that the porosity of the glomerular basement membrane is increased. Therefore, any change in the chemical composition of the diabetic basement membrane does not result in a structural defect that alters its role as a selective filter.

C. FUNCTIONAL DIABETIC MICROANGIOPATHY

The structural abnormalities characteristic of diabetic microangiopathy were demonstrated to occur at a later stage in the evolution of the disease [71]. Functional abnormalities were observed in several vascular beds prior to the appearance of any clinical signs of microangiopathy [30, 31, 54]. One manifestation of this functional microangiopathy is that alterations in blood flow precede structural changes in blood vessels.

1. Blood Flow Changes in Diabetes Mellitus

The gross blood flow has been studied in various vascular beds of human diabetics [2, 11, 18, 36, 38, 40, 55]. The vascular beds studied include the skin [36, 38], retina [55], abdominal subcutaneous adipose tissue [38, 40], and skeletal muscle in the forearm [11, 18, 38, 40] and calf [2]. The techniques used for flow measurement include isotope clearance [2, 36, 38, 40], fluorescein angiography [55], mercury-in-rubber strain gauges and venous occlusion plethysmography [2, 11, 18, 36, 38].

A high resting blood flow has been found in the retina [55] and forearm [11] of diabetics with no or only mild microangiopathy. Furthermore, it was demonstrated that the elevated blood flow declined towards normal as the microangiopathic lesions progressed to severe levels. Christensen [18] demonstrated that the resting forearm blood flow to be increased in newly diagnosed, untreated juvenile diabetics.

Alpert et. al. [2] used venous occlusion plethysmography to determine resting blood flow in normal persons and patients with prediabetes and diabetes with and without microangiopathy. No significant differences in resting calf blood flow were found between the groups. This finding does not support the work of Butterfield and Holling [13], Christensen [18] and Kohner et. al. [55]. It is supported by the work of Greeson et. al. [36]. Using plethysmographic techniques to study blood flow in the calf, they found no difference in blood flow between diabetics and normal controls.

In a comparison of the abdominal subcutaneous fat tissue blood flow in healthy individuals and in diabetics made be Haggendal et. al. [40], it was demonstrated that the average blood flow was higher in diabetics than in healthy individuals. Administration of insulin to the diabetics

lowered the blood flow. The relationship between elevated peripheral blood flow and metabolic control was investigated by Gunderson [38]. Previously insulin-treated patients, in whom insulin was withdrawn for a few days, showed elevated blood flow in the forearm, subcutaneous abdominal fat tissue and skin. A group of newly diagnosed, non-treated diabetic patients showed elevated blood flow only in the subcutaneous abdominal fat tissue and skin. During periods of good metabolic control, the diabetics had normal values for forearm, subcutaneous abdominal fat tissue and skin blood flow.

2. Changes in Blood Vessel Responsiveness

In conjunction with the altered blood flows, the responsiveness of diabetic blood vessels to various stimuli was found to be abnormal [18, 36, 40]. Greeson et. al. [36] demonstrated an impaired ability of diabetic cutaneous blood vessels to respond to dilating stimuli. If the resistance vessels were maximally dilated when the stimulus was applied, then the results could be falsely interpreted as a diminished vascular reactivity. Without quantitatively measuring the dimensions of the cutaneous vessels when the dilating stimuli was applied, no direct assessment of vascular reactivity can be made.

These studies were performed using plethysmographic techniques. This measures both skin and muscle blood flow. A technique that measures cutaneous blood flow independently of muscle blood flow would have been more appropriate for assessing the reactivity of cutaneous blood vessels to dilating stimuli. Greeson et. al. did use an isotope clearance technique to measure resting skin blood flow. They did not use this method to test the responsiveness of the cutaneous vasculature to dilating stimuli.

Christensen [18] investigated abnormal vascular function in a group of short-term diabetic patients. The rate of return of blood flow towards basal levels following reactive hyperemia was determined before treatment or after withdrawal of insulin and again after insulin treatment. In the diabetic patients peak flow was greater than in the non-diabetic controls. No significant difference was seen before or after insulin treatment. Reactive hyperemia was found to be considerably prolonged in the diabetic patients. This abnormality disappeared after insulin treatment.

The maximum blood flow in the anterior tibial muscle during reactive hyperemia following ischemia and exercise has been measured by various investigators with conflicting results. Trap-Jensen and Lassen [110] and Trap-Jensen et. al. [111] found no significant difference in the maximal hyperemic muscle blood flow within the groups (non-diabetic, recently diagnosed and long-term diabetics) studied. Using identical techniques, Alpert et. al. [2] found normal persons had hyperemic muscle flows significantly greater than those in prediabetics or diabetics with and without microangiopathy. No differences were found between the prediabetic and diabetic groups. The hyperemic muscle blood flows in the prediabetic and diabetic groups were identical with those reported by Trap-Jensen and Lassen [110] and Trap-Jensen et. al. [111] for their non-diabetic, recently diagnosed and long-term diabetic patients.

Qualitative descriptions of the bulbar conjuctiva in diabetics were carried out by Ditzel and his co-workers using the biomicroscope combined with a flash photographic technique [25, 26, 27, 28, 30]. The most consistent observation was the pathological formation of erythrocyte

aggregations. A reduction in the velocity of flow was associated with the formation of erythrocyte aggregates. This created a condition which favored microthrombus formation and deposition of carbohydrate-lipid-protein complexes in the walls of small blood vessels.

3. Summary of Changes in Blood Flow and Blood Vessel Responsiveness

The exact role of altered blood flow in producing microangiopathy is not clear. Only by adequately defining the degree of microangiopathy and the degree of metabolic control can this be established. In the gross blood flow studies the degree of metabolic control has been either abnormal [11, 18, 40] or not described [2, 36, 55]. In addition, the degree of microangiopathy was either not stated [18, 38] or diabetics without evidence of microangiopathy were grouped with diabetics with evidence of microangiopathy.

Vasodilatation and/or an elevated blood pressure could be responsible for the increase in blood flow observed in diabetics. As vessel dimensions and blood pressure were not quantitatively measured in these studies, the mechanisms responsible for the elevated blood flow are not known. The same arguments are applicable to the mechanisms responsible for return of blood flow towards normal in satisfactorily controlled diabetics and in diabetics with progressively worsening microangiopathy.

The exact relationship between altered blood flow, abnormal vascular reactivity and diabetic microangiopathy is not clear. The failure of investigators to define exactly the state of metabolic control and the presence of microangiopathic lesions has left the issue muddled. The investigations of Christensen [18] and Gunderson [38] definitely suggest insulin alters the ability of blood vessels to regulate their tone. No

investigations where direct quantitative measurements of blood flow in microvessels, i.e., arterioles, capillaries and venules have been conducted. Therefore, the relationships between the insulin-deficient state, abnormal vascular function and thickening of the capillary basement membrane remain speculative.

D. BIOCHEMICAL AND PHYSIOLOGICAL ALTERATIONS OF DIABETIC BLOOD

1. Changes in Serum Protein Composition

The blood vessels are not the only component of the circulatory system to be biochemically and physiologically altered in diabetes mellitus. The blood also undergoes biochemical and physiological alterations. In addition to hyperglycemia, there are changes in the plasma concentrations of the circulating protein-bound carbohydrates, proteins and lipids. Characteristically, a decrease in serum albumin, accompanied by an increase in the α- and β-globulins was observed [9, 27, 66, 69, 70, 88]. Winzler [127] has reviewed the considerable wealth of data in the literature substantiating this observation. It was suggested, but not proven, that these changes precede the degenerative alterations in blood vessels [88].

2. Abnormal Blood Viscosity in Diabetes Mellitus

The blood proteins impart a significant influence on the rheological characteristics of circulating blood [17, 67, 121]. Modifications in the plasma protein distribution may have considerable bearing on the viscous behavior of blood in the various microvascular compartments, i.e., arterioles, capillaries and small venules, as well as on the development of the degenerative changes in the smaller blood vessels of individuals with diabetes [69]. Blood viscosity can only be of importance in the development of microangiopathy if blood viscosity is altered before the occurence of microangiopathic lesions.

In vitro studies of the rheological properties of human diabetic blood and serum have yielded conflicting results, both in regards to changes in diabetes and about the relation of viscosity to diabetic microangiopathy. Cogan *et. al.* [19] first published evidence of serum viscosity elevation in diabetes mellitus. However, serum viscosity was not greater in diabetics with microangiopathy than in those without microangiopathy. Studies of diabetic patients without evidence of vascular complications by Ditzel [29] demonstrated no significant differences in whole blood viscosity between diabetic and non-diabetic patients. Similar studies with more accurate viscometers have indicated that blood and serum viscosity are both elevated in diabetics [19, 45, 67, 69, 70, 95]. The viscosity is even more strikingly increased in diabetics with evidence of microangiopathy [45, 67, 69, 70], and especially at low shear rates [43].

Over the years the increased serum viscosity might favor the development of diabetic microangiopathic lesions. Despite quantitative differences in the degree of viscosity elevation, the hyperviscosity syndromes share such features as retinal changes, neurological disorders and glomerular basement membrane thickening with diabetic microangiopathy [71, 119]. Microaneurysms, hemorrhages and retinal vein dilatation have been found in macroglobulinemia [4, 32] and myelomatosis [56]. Both are conditions in which the blood viscosity is raised. Microangiopathic lesions have been produced experimentally by increasing the blood viscosity with dextran [63].

3. Summary of Biochemical and Physiological Alterations of Diabetic Blood

Conclusions about the rheology of blood in the microcirculation can only be based on inferences from the physical characteristics of blood withdrawn from the macrocirculation but analyzed in an *in vitro* system which

may not represent the in vivo situation. Without knowledge of the shear stresses and shear rates existing in the microcirculation, extrapolation from the in vitro conditions of a viscometer to conclusions about the hemodynamic significance of viscosity in the microcirculation cannot be made. Silent testimony to the difficulty of making in vivo measurements of shear stress and shear rate for blood in the microcirculation is found in the absence of such data in the literature. However, information of this nature would derive from quantitative measurement of microvessel blood flow.

E. SUMMARY OF LITERATURE REVIEW AND STATEMENT OF PURPOSE

Thickening of capillary basement membranes has emerged as the fundamental morphologic alteration characteristic of diabetic microangiopathy [53, 92, 123, 124]. Although the microangiopathy of the the retina and kidneys has traditionally received great attention, recent studies have shown that most, if not all, capillary beds of the body are affected [30, 54, 71, 96, 107, 124]. The ubiquitous nature of diabetic microangiopathy suggests that the formation of microangiopathic lesions is relatively independent of extravascular metabolic processes. It is unlikely that an extravascular metabolic process unique to a single organ is responsible for the accumulation of PAS positive material in the vessel walls of arterioles, capillaries and venules. This indicates a change in the blood may play a role in the development of diabetic microangiopathy.

Changes in plasma protein composition are known to occur in diabetes mellitus [127]. Both increased plasma viscosity and enhanced intravascular aggregation have been related to the same changes in plasma protein composition [27, 69, 70]. The circulatory problem in diabetes mellitus is

confined mainly to the capillaries and small venules; hence, this is the level of the circulation where intravascular rheology requires analysis in this disease. Therefore, information about diabetic microangiopathy will result from techniques which provide for direct *in vivo* quantitation of blood flow in microvessels.

The purpose of this study was to evaluate quantitatively the microcirculation in an animal model of diabetes mellitus. Specifically, the red cell velocity in mesenteric microvessels, i.e., arterioles, capillaries and venules, were measured in non-diabetic and streptozotocin-diabetic rats.

CHAPTER III

METHODS

A. ANIMALS AND CARE

Adult male Holtzman rats (>60 days old) were used in this study. They were acclimated to the temperature (76±2 ^{o}F) and the light period (7 AM to 7 PM) in the animals quarters for at least one week before use. Laboratory chow and water were provided ad libitum. Prior to an experiment, the animals were fasted overnight. This consisted of removing food, but not water, from the animals at approximately 6 PM on the evening immediately preceding an experiment.

B. INDUCTION OF DIABETES MELLITUS AND EVALUATION OF THE DIABETIC STATE

Streptozotocin (Upjohn Co., Kalamazoo, Michigan) was used to induce diabetes mellitus in fed male Holtzman rats. The rats were anesthetised with sodium pentobarbital (1.6 mg, i.p.) to facilitate rapid handling. A solution of streptozotocin (100 mg/dl) in citrate buffer (0.1 M in 0.9% saline) was made not more than 2.5 minutes before an intravenous injection of streptozotocin (25 mg) was given into the dorsal vein of the penis. At the time of injection these rats were 12 weeks old and of a mean body weight of 330 grams.

The polydipsia, polyphagia and polyuria evident in these rats during the weeks following the streptozotocin treatment as well as their weight loss and emaciated appearance suggested a diabetic state. Only those rats whose plasma glucose concentration following an overnight fast was greater than 140 mg/dl were used. A Yellow Springs Instrument Model 23A Glucose

Analyzer was used to measure plasma glucose concentrations. The blood sample was obtained from the left carotid artery on the day of the experiment.

The effect of streptozotocin-diabetes on the microcirculation was assessed by measuring the red cell velocity in the mesenteric microvessels of three groups of rats. One group of rats was not treated with streptozotocin. They served as age-matched, normal controls. The streptozotocin-treated animals were divided into two groups: a short-term and a long-term diabetic group. The rats in the short-term diabetic group served as hyperglycemic controls. Experiments conducted on control animals were interspersed with those conducted on the streptozotocin-treated animals.

C. SURGICAL PROCEDURE

Fasted male Holtzmann rats were anesthetized with sodium pentobarbital (35 mg/kg, i.m.). The abdomen and ventral aspect of the neck were shaved in preparation for surgery.

The right jugular vein was cannulated (PE-50, Clay-Adams) for the intravenous administration of maintenance doses of anesthesia. A second PE-50 catheter was placed in the left carotid artery and coupled to a Statham P23Gb pressure transducer for arterial pressure measurement.

The trachea was cannulated and the ventilation of the animal was controlled with a Harvard Apparatus Rodent Respirator. Respiration was maintained at 60 breaths per minute. The tidal volume of 2-3 ml was adjusted for each animal according to the ventilation graph accompanying the respirator.

A 3" X 5" piece of surgical drape was adhered to the abdomen. The surgical drape prevented loose hair clippings from getting on the exte-

riorized mesentery. A 2" midline incision, centered on the umbilicus, was made through the drape and into the peritoneal cavity along the linea alba.

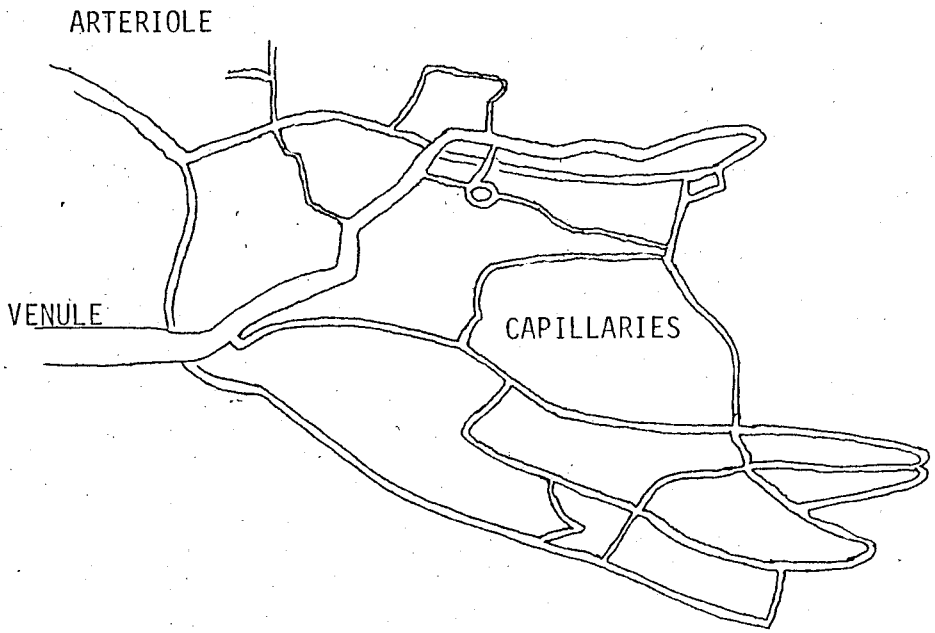
Each animal was placed on its right side in a holder on a specially designed, constant temperature (37 ^{o}C), water-heated, plexiglass microscope stage. Cotton-tipped swabs moistened with Tyrode's solution were used to gently remove a short length of ileum from the abdominal cavity. Once exteriorized, the mesentery was loosely draped over the viewing pedestal on the plexiglass stage. A standing solution of warm (37 ^{o}C) Tyrode's solution (composition in mM: NaCl 142.5; KCl 2.7; $CaCl_2$ 1.4; $MgCl_2$ 0.5; $NaHCO_3$ I1.9; NaH_2PO_4 0.3 and glucose 5.5) adjusted to 300±5 mOsm with NaCl and to pH 7.4 with HCl and NaOH was used to keep the tissue moist. A small piece of polyvinyl film (Saran Wrap) was used to cover the mesentery to retard evaporation, yet permit observation.

D. SELECTION OF VESSELS

A section of the mesenteric microcirculation consisting of a vascular loop of an arteriole, capillaries and venules was selected for the measurement of red cell velocity. A schematic representation of such a loop is shown in Figure 1. The following criteria served to establish the normalcy of the microcirculation in each preparation: (1) flow through the terminal arterioles was sufficiently rapid so that individual blood cells could not be identified, (2) the collecting venules had a steady, continuous forward flow without evidence of retrograde flow or oscillation, and (3) mean arterial perfusion pressure as measured in the left carotid artery was not less than 70 mm Hg. Thirty-four percent of the animals were discarded because according to the criteria the preparation was aberrant.

FIGURE 1

MESENTERIC MICROCIRCULATION



This is a diagramatic representation of the terminal vasculature in the mesenteric microcirculation. It consists of a vascular loop of an arteriole, capillaries and the effluent venule. Such vascular loops were selected for the measurement of red cell velocity.

A number of changes occurred in the vasculature which indicated tissue deterioration and were used as criteria for terminating experiments. The changes generally occurred in the following order: (1) the appearance of leukocytes sticking to or rolling along the walls of the collecting venules, (2) extravasation of red cells either by diapedesis or petechial hemorrhage, and (3) extensive intravascular coagulation.

E. MICROSCOPE OPTICS

The plexiglass stage which held the rat and the exteriorized mesentery was placed on the mechanical stage of a Leitz Panphot microscope. A xenon lamp (XBO 150W) was used to transilluminate the mesentery. A magnified image of the mesenteric microvasculature was projected onto a viewing screen by a projection prism (6.3 X) and two Leitz objectives (UM 20/0.33). One of the objectives was used as a long working distance condensor and the second was used as a high, dry objective. The total magnification realized at the viewing screen was 237 X.

F. MEASUREMENT OF VESSEL DIMENSIONS

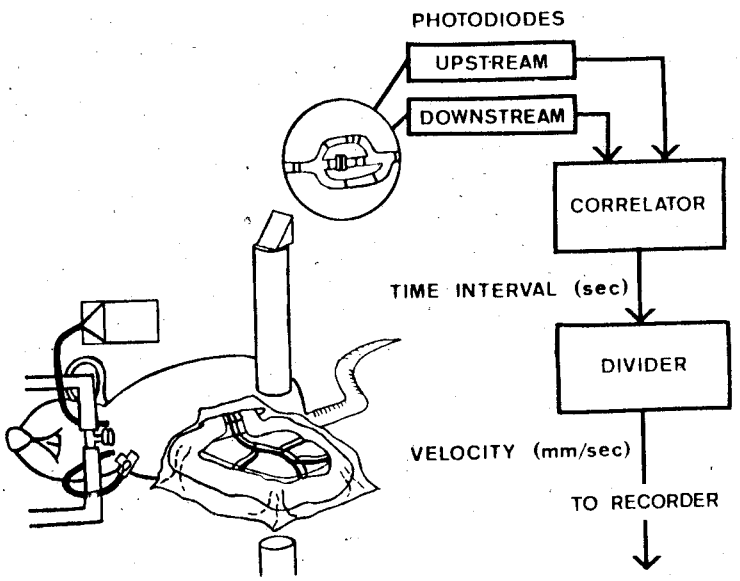
The image of a Bausch and Lomb stage micrometer was projected onto the viewing screen. The distance between the minor divisions (10 microns) was drawn onto the viewing screen. This graticule was used to measure the outside diameter of all vessels from which velocity recordings were made.

G. MEASUREMENT OF RED CELL VELOCITY

A modification of the dual-slit photometric technique of Wayland and Johnson [117] was used to measure red cell velocity. The image of a microvessel was aligned perpendicular to two parallel slits in a viewing screen as shown in Figure 2. As the image of a red cell passed in front

FIGURE 2

EXPERIMENTAL ARRANGEMENT
FOR ON-LINE MEASUREMENT OF
RED CELL VELOCITY



Schematic diagram of preparation and on-line red blood cell velocity measuring system. Image of microvessel is projected onto a screen containing dual slits. Upstream and downstream photodiodes are time correlated and the resulting time interval is divided into the distance between the slits to obtain red cell velocities.

of the slits, the voltage output of a photodiode (United Detector Technology, Inc., PIN 020A) mounted behind each slit varied. When the vessel image was properly aligned, the magnitude of this variation was identical for both detectors, differing only in the time it took for the image of the red cell to travel the distance between the two slits. This transit time was determined by an on-line cross-correlation technique [46, 118]. An analog divider circuit, programmed with the distance between the photodetectors computed the red cell velocity.

A strip chart recorder (Offner Type R dynograph) was used to record continuously the red cell velocity and arterial pressure. Red cell velocity data was collected and tabulated at two or five second intervals over the observation period. These were used to calculate the average red cell velocity in the vessel.

H. STATISTICAL ANALYSIS

Homogeneity of variance was tested for by the F-maximum test [97]. One way analysis of variance was used to analyze for significant differences between corresponding variables of the three experimental groups (control, short-term and long-term streptozotocin-diabetics). The Scheffe contrast was used to determine which groups differed. Correlation coefficients were calulated according to the methods of Pearson's (r) [23]. The 5% level of significance was used.

CHAPTER IV

RESULTS

A. CONFIRMATION OF DIABETES MELLITUS

The plasma glucose concentrations and body weights of the control, short-term and long-term streptozotocin-treated animals are shown in Table I. At the time of streptozotocin injection the fed weight of the 59 treated animals was 329 ± 4.8 grams. All values are means ± standard errors. Those animals used one week after the streptozotocin injection weighed 276 ± 9 grams. This was an average weight loss of 53 grams. There was a more gradual weight loss during subsequent weeks. The treated animals weighed an average of 266 ± 8.6 grams 25 days after the streptozotocin injection.

In addition to the weight loss, the streptozotocin-treated animals exhibited polydipsia, polyphagia and polyuria. This is consistent with an induced diabetic state and was confirmed by the elevated plasma glucose levels in these animals. The plasma glucose concentrations of the non-treated animals was 82 ± 7 mg/dl. At the end of one week of diabetes mellitus, the plasma glucose concentration was 273 ± 30 mg/dl. As the duration of diabetes increased, this rose to 296 ± 36 mg/dl in the long-term diabetic animals.

B. EFFECT OF STREPTOZOTOCIN DIABETES ON RED CELL VELOCITY

The red cell velocity in the various compartments of the microcirculation is shown in Figure 3. This figure represents data from viable mesenteric preparations of seven control (C), nine short-term (S) and seven long-term (L) diabetic animals.

TABLE I

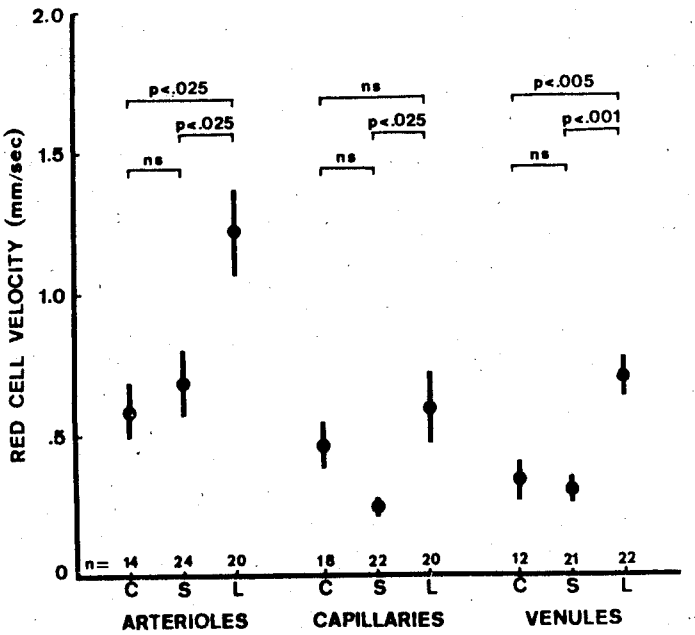
PLASMA GLUCOSE CONCENTRATIONS IN
CONTROL AND STREPTOZOTOCIN-TREATED ANIMALS

	CONTROL	STREPTOZOTOCIN-TREATED SHORT-TERM	STREPTOZOTOCIN-TREATED LONG-TERM
NUMBER OF ANIMALS (N)	15	12	12
AGE (days)	96 ±3	89 ±2	105 ±3
WEIGHT (grams)	353 ±11.4	276 ±9	266 ±8.6
DURATION OF DIABETES (days)	0	7 ±0.5	25 ±3
PLASMA GLUCOSE (mg/dl)	82 ±7	273 ±30	296 ±36

The 24 streptozotocin-treated animals are those animals which survived the diabetes which developed following the injection of streptozotocin. The duration of diabetes refers to the time since the streptozotocin injection.

FIGURE 3

EFFECT OF STREPTOZOTOCIN DIABETES ON
RED CELL VELOCITY



Red cell velocity (mean ± SEM) in arterioles, capillaries and venules of 7 control (C), 9 short-term (S) and 7 long-term (L) diabetic animals. The n is the number of vessels observed. The level of significance is indicated by the P values; ns indicates $P > 0.05$.

No statistically significant difference was found between the red cell velocities measured in the various microvessels of the control and short-term diabetic animals. The average red cell velocity in the 14 control arterioles studied was 0.60 ± 0.10 mm/sec as compared with 0.68 ± 0.12 mm/sec in the 24 short-term diabetic arterioles. In the 18 control capillaries, the red cell velocity was 0.45 ± 0.09 mm/sec versus 0.24 ± 0.03 mm/sec in the 22 short-term diabetic capillaries. The velocity at which red cells moved through the 12 non-diabetic venules was 0.34 ± 0.07 mm/sec. It averaged 0.30 ± 0.07 mm/sec in the 21 short-term diabetic venules.

The red cell velocity of the 20 arterioles studied in the long-term animals was 1.22 ± 0.15 mm/sec as compared with 0.68 ± 0.12 mm/sec for the short-term diabetic animals and with 0.60 ± 0.10 mm/sec for the control animals. The red cell velocity in the arterioles of the long-term diabetic group was significantly greater ($P < 0.025$) than in the short-term diabetic or control animals.

The red cell velocity in the 20 capillaries studied in the long-term diabetic animals was 0.59 ± 0.12 mm/sec as compared with 0.45 ± 0.09 mm/sec for the control animals and with 0.24 ± 0.03 mm/sec for the short-term diabetic animals. The red cell velocity in the capillaries of the long-term diabetic animals was significantly greater ($P < 0.025$) than in the short-term diabetic animals, but was not significantly greater than in the control animals.

The red cell velocity in the 22 venules studied in the long-term diabetic animals was 0.70 ± 0.08 mm/sec as compared with 0.34 ± 0.07 mm/sec for the control animals and 0.30 ± 0.04 mm/sec for the short-term diabetic

animals. The red cell velocity in the venules of the long-term diabetic animals was significantly greater than in the control animals ($P < 0.005$) or the short-term diabetic animals ($P < 0.001$).

An average for the red cell velocity in each segment of the microcirculation was determined for each preparation. The correlation between the severity of diabetes, as judged by the plasma glucose concentration, and the red cell velocity in the arterioles, capillaries and venules was determined by calculating the Pearson (r) moment correlation coefficient. The correlation coefficients are shown in Table II. No significant correlation was found between the plasma glucose concentration and the red cell velocity in any segement of the microcirculation.

C. EFFECT OF STREPTOZOTOCIN DIABETES ON VESSEL DIMENSIONS

The caliber of the microvessels in the various compartments of the microcirculation of the same control (C), short-term (S) and long-term (L) animals is shown in Figure 4. This represents data from the same arterioles, capillaries and venules in which the red cell velocity was measured. The outside diameter of the arterioles in the control (C), short-term (S) and long-term (L) diabetic animals was 12.5 ± 0.8 microns, 15.2 ± 1.4 microns, and 12.1 ± 0.8 microns, respectively. These measurements were not significantly different. The diameter of the capillaries in these animals was 7.9 ± 0.4 microns, 7.7 ± 0.4 microns and 7.7 ± 0.5 microns, respectively. These measurements did not differ significantly. The caliber of the venules in the same control (C), short-term (S) and long-term (L) diabetic animals was found to be 22.8 ± 2.1 microns, 17.9 ± 1.8 microns and 17.5 ± 1.4 microns, respectively. Again, there was no statistical difference among these groups.

TABLE II

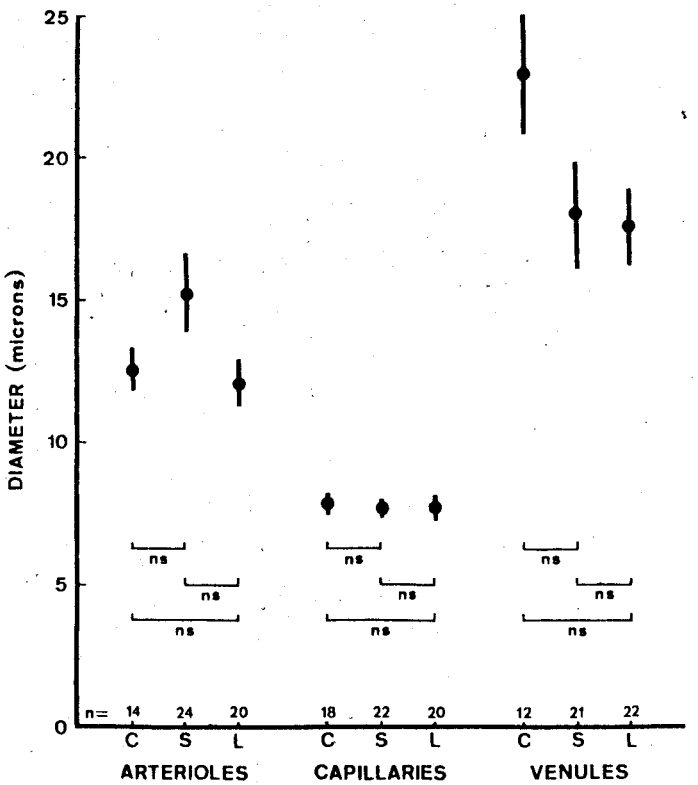
CORRELATION BETWEEN PLASMA GLUCOSE CONCENTRATION AND RED CELL VELOCITY

	ARTERIOLES	CAPILLARIES	VENULES
CORRELATION COEFFICIENT (r)	0.29	-0.07	0.08
DEGREES OF FREEDOM (df)	18	17	17
LEVEL OF SIGNIFICANCE	P > 0.10	P > 0.10	P > 0.10

This table shows the Pearson (r) moment correlation coefficient between the plasma glucose concentration and the red cell velocity in the arterioles, capillaries and venules of the non-diabetic and diabetic animals. The degrees of freedom and the level of significance are also indicated.

FIGURE 4

EFFECT OF STREPTOZOTOCIN DIABETES ON
VESSEL DIMENSIONS



Outside diameter (mean ± SEM) of arterioles, capillaries and venules of 7 control (C), 9 short-term (S) and 7 long-term (L) diabetic animals. The n is the number of vessels observed. The level of significance is indicated by the P values; ns indicates $P > 0.05$.

D. EFFECT OF STREPTOZOTOCIN DIABETES ON SYSTEMIC BLOOD PRESSURE

Simultaneous with the measurement of red cell velocity in the various microvascular segments was the recording of blood pressure in the left carotid artery. The average mean blood pressure was calculated for the period of red cell velocity measurement. These data are shown in Figure 5.

When the red cell velocity was being measured in the arterioles of the control (C), short-term (S) and long-term (L) diabetic animals, the blood pressure in the left carotid artery was 150 ± 4 mm Hg, 134 ± 4 mm Hg and 137 ± 3 mm Hg, respectively. There was a significant difference ($P < 0.025$) between the blood pressure in the control (C) and short-term (S) diabetic animals when arteriolar red cell velocities were being measured. No other differences were found.

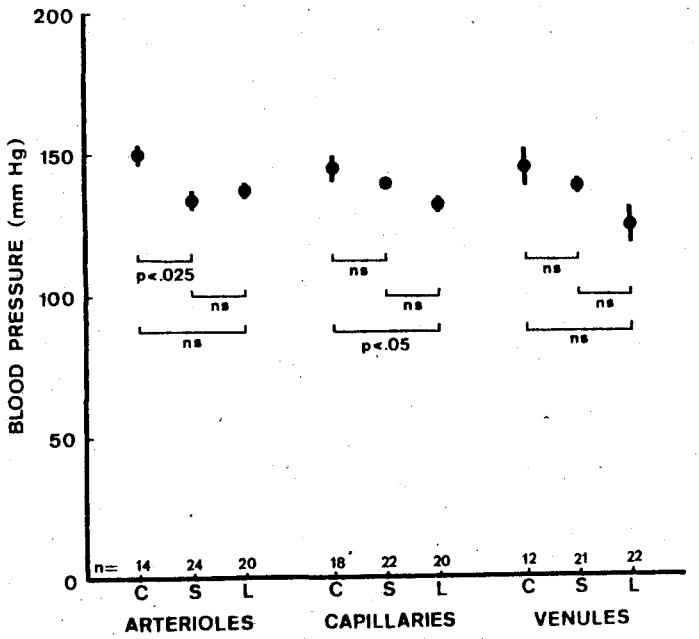
When the red cell velocity was being measured in the capillaries, the blood pressure was 146 ± 5 mm Hg in the control (C) animals as compared with 141 ± 3 mm Hg in the short-term (S) diabetic animals and 133 ± 3 mm Hg in the long-term (L) diabetic animals. The only significant difference ($P < 0.05$) was found between the control and long-term animals.

The blood pressure in the control (C), short-term (S) and long-term (L) diabetic animals was 146 ± 6 mm Hg, 139 ± 3 mm Hg and 131 ± 3 mm Hg, respectively, when the red cell velocity was being measured in the venules. There was no significant difference between these values.

From the data presented in Figure 5, it appears as if there is a trend for blood pressure to decrease as the duration of diabetes lengthens. An average mean blood pressure was determined for each preparation. Computation of the Pearson (r) correlation coefficient ($r = -0.29$; $df = 21$) for these data indicates no significant relationship exists between the duration of

FIGURE 5

EFFECT OF STREPTOZOTOCIN DIABETES ON
SYSTEMIC BLOOD PRESSURE



Mean systemic blood pressure (mean ± SEM) in the left carotid artery of 7 control (C), 9 short-term (S) and 7 long-term (L) diabetic animals measured at the same time red cell velocities were being recorded. The n is the number of vessels observed. The level of significance is indicated by the P values; ns indicates $P > 0.05$.

diabetes and systemic blood pressure. The correlation between systemic blood pressure and plasma glucose concentration ($r = -0.15$; $df = 19$) indicates that the apparent tendency for blood pressure to decrease as the duration of diabetes increased is not related to the plasma glucose concentrations.

E. EFFECT OF STREPTOZOTOCIN DIABETES ON LYMPHATIC ACTIVITY

In the course of an experiment, a lymphatic vessel would occasionally come into view. When this happened, the rates of contraction were measured. The relaxed and contracted diameters of the lymphatic vessels were also measured. These data are shown in Table III.

Lymphatic vessels were seen in only two non-diabetic (C) animals. This was because the mesenteric fat pads hid them from view. When the mesenteric fat pads atrophied, the lymphatic vessels were exposed. Seven lymphatics were seen in four short-term (S) and six lymphatics were seen in four long-term (L) diabetic animals.

The contraction-relaxation cycles appear most rapid in the larger collecting channels (150-250 microns). The periodicity varied from 7-33/min.

TABLE III

LYMPHATIC ACTIVITY
IN NON-DIABETIC AND STREPTOZOTOCIN DIABETIC RATS

GROUP	DIAMETER (microns) RELAXED	CONTRACTED	CONTRACTIONS PER MINUTE
C	175	100	28
C	130	110	18
S	250		0
S	150		0
	225		0
S	120	90	24
	140	110	22
	210	130	7
S	75	50	23
L	150	120	9
	200	175	12
L	250	200	10
L	200	150	24
	200	150	33
L	150	125	14

This table shows the relaxed and contracted diameters of collecting lymphatics in 2 control (C), 4 short-term (S) and 4 long-term (L) diabetic animals, along with their corresponding rate of contraction. In two of the short-term animals, the lymphatics showed no spontaneous contractions, hence it is not known whether the diameter measured represents a relaxed or contracted diameter.

CHAPTER V

DISCUSSION

A. INDUCTION OF DIABETES MELLITUS AND EVALUATION OF THE DIABETIC STATE

The purpose of this study was to quantitatively examine the effects of diabetes mellitus on circulatory dynamics within a microvascular network. This was achieved by measuring the red cell velocity in the mesenteric microcirculation of an animal model of diabetes mellitus.

Currently, streptozotocin is the drug of choice for the induction of experimental diabetes in laboratory animals [3, 49, 50]. The streptozotocin treated animals exhibited a weight loss when compared with age-matched controls. In addition, they exhibited polyphagia, polydipsia, and polyuria. This is consistent with an induced state of diabetes mellitus.

The diabetic state was confirmed by the elevated plasma glucose levels in these animals. The plasma glucose concentration in the short-term diabetic animals was 273 ± 30 mg/dl and was 296 ± 36 mg/dl in the long-term diabetic group, as compared with only 82 ± 7 mg/dl in the non-treated control group. These values are in agreement with values reported in the literature [3, 49, 50] for glucose levels in rats treated with comparable doses of streptozotocin and after similar periods of time had elapsed.

B. MICROCIRCULATION IN THE INTESTINAL MESENTERY

Unlike structures, such as skeletal muscle, which require considerable handling, the intestine can be exposed for study with a minimum of handling. The mesentery provides in a two-dimensional array all the elements of the

terminal vascular bed. It provides clear visualization of the vessel wall and blood cellular elements. The thinness and vascularity of the mesentery make it a valuable structure for transillumination. This makes it possible to use a dual-slit photometric system for measuring red cell velocity [117].

The ubiquitous occurence of diabetic microangiopathy throughout the body makes it unlikely that an extravascular metabolic influence unique to a single organ is responsible for the accumulation of PAS-positive material in the vessel walls of arterioles, capillaries and venules. This suggests that an intravascular derangement may be responsible for the development of microangiopathic lesions.

The metabolic influence of the loose connective tissue in the mesentery on the autoregulatory behavior of the terminal vascular bed is minimal. Flow through the mesenteric microcirculation is strongly regulated by myogenic mechanisms [48]. The mesenteric microcirculation is tailor made for investigating the influence of diabetes mellitus on microcirculatory phenomena. The results obtained would not be distorted by changes in the metabolic environment of the vascular bed peculiar to the influence of diabetes mellitus on a parenchymatous organ.

The most striking thing about the appearance of the diabetic mesentery was the marked atrophy of the mesenteric fat cells. The decrease in the size of the fat cells is consistent with the increased mobilization of fatty acids in diabetes mellitus.[122]. The fat cells were very prominent in the non-diabetic animals. The microvessels were clearly visible only in a narrow zone along the margin of the fat pads. Larger arterioles and venules could be less clearly discerned through the overlying translucent adipocytes. In the diabetic animals it was as if the adipocyte quilt had been thrown

back, clearly exposing the complete distribution and branching pattern of the microvessels lying in the mesenteric bed. The vessels brought to view were probably already in existence at the time diabetes was induced [98]. No gross anatomical differences in the appearance of the mesenteric microvascular architecture were observed.

The preparations used in this series of experiments were physiologically sound. The flow in all the arterioles was sufficiently rapid so that individual blood cells could not be identified. Changes of red cell velocity in the capillaries were irregular and unpredictable. The capillaries studied were all of the steady type in which individual red cell velocity measurements fluctuated randomly around a stable mean. Changes in the direction of movement, as well as in the number of capillaries with an active circulation were seen. The effluent flow by way of the post-capillary venules had a steady continuous forward movement with no evidence of retrograde oscillation.

Experiments conducted on control animals were interspersed with those conducted on diabetic animals. The microvessels studied in all the animals were taken from the same general area of the mesentery. The same criteria for assessing the initial viability of the preparations and the degree of deterioration were used in all the experiments. It is unlikely that bias in the selection of vessels or differences in the viability of the preparations are responsible for the differences observed in red cell velocity.

C. INFLUENCE OF STREPTOZOTOCIN ON RED CELL VELOCITY

The red cell velocity constitutes a good index of blood flow velocity which faithfully reflects all natural or induced changes of flow as shown

in the studies of Wayland and Johnson [117] and Intaglietta et. al. [46]. The red cell velocity in the arterioles, capillaries and venules of the control animals (Figure 3) was 0.60 ± 0.10 mm/sec, 0.45 ± 0.09 mm/sec and 0.34 ± 0.07 mm/sec, respectively. These results are comparable to those reported in the literature [10, 46, 83, 84] and with previous results obtained in this laboratory (unpublished observations).

The short-term diabetic animals served as hyperglycemic controls. Blood sugar levels per se have been demonstrated to have no effect on blood rheology [13]. Therefore, no difference in red cell velocity was expected to be found between the control and short-term diabetic animals. As anticipated, there was no significant difference between the red cell velocity measured in the microcirculation of the control animals and the red cell velocity measured in the arterioles (0.68 ± 0.12 mm/sec), capillaries (0.24 ± 0.03 mm/sec) and venules (0.30 ± 0.04 mm/sec) of the short-term diabetic animals.

The red cell velocity in all segments of the microcirculation of the long-term diabetic animals was greater than that measured in either the control or the short-term diabetic animals. The red cell velocity in the arterioles (1.22 ± 0.15 mm/sec) was more than twice that of the control animals and 1.8 times greater than that of the short-term diabetic animals. In the capillaries of the long-term diabetic animals, the red cell velocity (0.60 ± 0.12 mm/sec) was 1.3 times that of the non-diabetic animals and 2.5 times that of the short-term diabetic animals. Similar increases of red cell velocity were seen in the venular segment. The red cell velocity in this segment of the microvascualture was 0.70 ± 0.08 mm/sec in the long-term diabetic animals. This was more than double the red cell

velocity in the vessels of the non-diabetic animals and 2.4 times that of the short-term diabetic animals.

Since an increased red cell velocity was observed in the mesentery after one month of diabetes, it is a logical assumption that this happens in other vascular beds as well. More importantly, the changes in red cell velocity took place before thickening of capillary basement membranes has been reported to occur in chronically streptozotocin diabetic rats [12].

The increased red cell velocity observed in this study also is consistent with the hyperemic conditions observed in various tissues of diabetic patients [2, 11, 18, 36, 38, 40, 55]. Blood flow to an organ can be increased by only three mechanisms. These are: (1) an increase in blood pressure, (2) an increase in the cross-sectional area of the resistence vessels, and (3) a decrease in viscosity. As these variables were not measured in the diabetic patients, it is not possible to define clearly the mechanism responsible for their elevated flow.

D. SUGGESTED MECHANISMS FOR THE INCREASED RED CELL VELOCITY FOUND IN STREPTOZOTOCIN RATS

Because the blood pressure of the long-term diabetic animals was not greater than that of the control or short-term diabetic animals, the increased red cell velocity found in the long-term diabetic rats cannot be explained on the basis of this parameter. In order to account for the increased red cell velocity on the basis of vessel dimensions alone, the measured diameters would have to be 5 to 8 microns larger. (Equation 4.7 on page 136 of Charm and Kurland [14] was used to make this calculation.) In none of the microvascular segments did the outside diameter differ significantly from group to group. An increase of the inside radius of the

blood vessel not reflected in a larger outside diameter could account for a portion of the increased red cell velocity in the long-term diabetic group. The magnitude of the diameter change required and the thickness of the vessel walls at this level of the microcirculation makes this unlikely.

A decrease in viscosity to half of normal values would result in a doubling of blood flow. In vitro rheological studies of blood and serum have demonstrated that the viscosity of blood and serum is elevated in diabetics with evidence of microangiopathy [19, 45, 67, 69, 70, 95], and more so at low shear rates [45]. There has been one report of decreased serum viscosity in a group of diabetic patients [75]. Although the Ostwald viscometer employed by Mosora et. al. [75] appears to be a simple device, its use is subject to a number of errors [15] that make it inappropriate for quantitative biorheological measurements. This observation of decreased serum viscosity must be discounted until a more detailed report is published clearly defining the metabolic condition of the patients and the presence or absence of microangiopathy.

The relationship between the viscosity and the shear rate of blood is such that viscosity increases as the shear rate is reduced towards zero (shear thinning) and viscosity at high shear rates is essentially independent of shear [17]. A wide range of shear rates exist within the vascular compartments of the circulation. It is estimated that the lowest shear rates are to be found in the post-capillary venules [16, 89]. If the shear-dependent region is encountered in the venules, then the viscosity of blood would be preferentially elevated in this vascular compartment. The expected response in the venules to a shear-dependent increase in viscosity

would be sluggish flow. Such a response in the post-capillary venules would be even more pronounced if the degree of shear-dependence of blood viscosity is enhanced [45].

As blood traverses the microcirculation it does not flow as a homogeneous suspension as it does in larger vessels; rather, it flows as a two phase system--cells and plasma. Plasma alone is the liquid phase and plasma viscosity is the significant viscosity parameter. Blood viscosity, per se, is of little importance when considering microcirculatory flow [14, 47]. Chien [17] has developed a theory of blood viscosity that is especially applicable to the microvasculature where flow is of two distinct phases (cells and plasma) rather than bulk flow is taking place. This theory of blood viscosity is based on the concept of effective cell volume, which is defined as the sum of the cell volume and the volume of the surrounding fluid that behaves as if it were a rigid extension of the cell.

Red cell velocity exceeds plasma velocity in blood flow through small diameter vessels [106]. The difference between the velocity of plasma and the velocity of red cells is accentuated by increases of blood [85] or plasma [86] viscosity. Rosenblum [85, 86] observed that when the fluorescein transit time through the cerebral microcirculation of mice was lengthened because of increased plasma viscosity, erythrocyte velocity measured by ultra-high microcinematography was within normal limits or elevated. Rosenblum's results are consistent with Chien's theory of blood rheology [17]. According to the concept of effective cell volume, an increase in plasma viscosity would increase cell deformation and thereby decrease the effective cell volume. This would result in a decrease in the relative apparent viscosity of blood. An increase in red cell velocity would result.

It is possible that a shear-dependent increase in plasma viscosity occurred in the long-term diabetic animals. The increased red cell velocity in the arterioles and venules of the long-term diabetic animals is consistent with Rosenblum's observation of normal or elevated erythrocyte velocity in animals with an elevated plasma viscosity. Valid conclusions about plasma viscosity, the influence of plasma viscosity on red cell deformation and red cell velocity cannot be reached by measuring red cell velocity alone. An in vitro assessment of the viscosity of plasma withdrawn from animals in which the red cell velocity has been measured is necessary in order to reach such conclusions.

The red cell velocity in the venules of 3 of the 9 short-term diabetic animals was less than 60% of the group mean (0.30 ± 0.04 mm/sec). In only one of these animals was intravascular erythrocyte aggregation observed when the preparation was still considered viable. The aggregates were observed only in the venular segment of the microcirculation. They were small, consisting of only three or four cells clumped together. They did not distort or plug the vessel. Aggregation not related to preparation deterioration was observed in only one other short-term diabetic animal. The red cell aggregates were distinctly visible in the venular segment. They did not distort or plug the venules. The velocity of the red cells in the venules of this preparation averaged 0.38 mm/sec, slightly faster than the average velocity for this group.

No aggregations of red cells not attributable to preparation deterioration were observed in the microcirculation of the long-term diabetic animals. This may be due to the disaggregating shear forces of the blood flow being greater than the adhesive forces acting between red cells.

Therefore aggregates were never formed. It may also be that aggregates were formed, but because of their velocity and the flicker-fusion characteristics of the human eye, they were not distinctly visible. Use of an optical recording system with sufficient resolution that permits review of the observations is necessary in order to adequately determine the extent of red cell aggregation under conditions of increased red cell velocity in these animals.

In the post-capillary venules with diameters slightly larger than the major diameter of red blood cells, red cell aggregation can only be in the form of linear rouleaux with its axis aligned with flow. Under such a geometric limitation, the theory of blood flow presented by Chien [17] predicts a reduction in effective cell volume and a decrease in the apparent viscosity of blood. If aggregates did form in the post-capillary venules, the expected response would be an increase in velocity. The elevated red velocity measured in the post-capillary venules of the long-term diabetic animals is consistent with this micro-rheological theory of Chien.

Both increased plasma viscosity and enhanced intravascular aggregation have been related to changes in plasma protein composition [27, 68, 69]. There is a decline in the plasma concentration of albumin and a concomitant increase in that of α-glycoproteins. These proteins are produced by liver parenchymal cells. Thus a change in the liver protein synthesis pattern appears important in the development of diabetic microangiopathy. The degree of diabetes required to disrupt liver metabolism is not known [69]. It may be that the diabetes mellitus in the animals used in these experiments was not severe enough and/or of long enough duration to result in the disruption of liver metabolism and produce these changes in plasma proteins.

Streptozotocin has been used to produce diabetes mellitus of graded severity in laboratory animals [49, 50]. A streptozotocin diabetic animal can be used to analyze the relationship between depleted pancreatic immunoreactive insulin, alterations in liver protein synthesis patterns and deranged carbohydrate metabolism. Such an animal model would give information about the duration and severity of diabetes necessary to disrupt liver metabolism. This animal model would give information about the degree of diabetes necessary to produce changes in the rheological properties of blood and plasma. An in vitro assessment of blood and plasma viscosity could be made and compared with alterations in the composition of the plasma proteins.

The fluid mechanical processes which occur during the movement of an erythrocyte through the lumen of a capillary are very complex. They include the interaction of a deformable red cell with the luminal surface of the capillary wall. The nature of the endothelial layer and its possible effects on the determination of microcirculatory flow dynamics have not been determined [37].

The blood in the microcirculation is not in direct contact with the endothelial cells, but rather with the layer or film adherent to them. Luft [117] has electron micrographic evidence for the existence of a layer lining the lumen of blood vessels. Copley and others [20, 22, 77, 109] have postulated the endo-endothelial lining of blood vessels consists of a fibrin film which is dynamically maintained and controlled by fibrinolytic processes in the marginal plasma layer.

It has been observed that tubes coated with fibrin offer less resistance to blood and plasma flow than uncoated tubes [20]. The physical mechanism responsible for the decreased resistance to blood flow in fibrin

coated tubes is not known [37]. One possibility is the effect of the electric charge on the red cell and vessel wall. Most cells are negatively charged and the negatively charged tubing affords lower flow resistance to red cells [15]. Oka has suggested that the apparent viscosity of blood and plasma will always show a decrease when blood is in contact with negatively charged substances [77].

Electrokinetic phenomena arise when movement occurs along the solid/liquid interface of a charged system [90, 91]. This condition is duplicated in the blood vessels of all living animals. The role of electrical forces in the vascular system has not been readily accepted except for potentials measured in connection with contraction of the heart and of vascular smooth muscle. The hemodynamics of the vascular system have overshadowed the electrical properties of the surfaces of blood cells and of the endothelial lining of blood vessels. The in vivo and in vitro data relative to the negative charge on the components of the vascular system suggest that electrokinetic phenomena are significant when flow in the microcirculation is being considered [15, 41, 87]. Alterations in the charge carried by any of the components of the vascular system may be of significance as regards the interactions which occur between components of the system.

There is no reason to believe that reactions which alter the basement membrane will not have a comparable action on the layer lining the lumen of blood vessels. The use of ruthenium red to stain the endo-endothelial layer would show whether the fine structure of this layer is altered in diabetes [21, 61]. The importance of changes in the endo-endothelial layer for microcirculatory hemodynamics could be obtained from

cross-perfusion studies. A segment of intestine and mesentery could be isolated from a streptozotocin-diabetic animal and perfused with blood from a non-diabetic animal. The red cell velocity could be measured in such a preparation.

It is conceivable that a change in the electrical characteristics of the vessel wall could influence transcapillary fluid exchange via electro-osmotic processes. Such changes could also result in the collection of fibrin at the luminal surface of the endothelium. The net effect would be the increased deposition of fibrin and other plasma proteins in the walls of the blood vessels. These fibrinous deposits, being remote from fibrinolytic processes in the vessel wall, undergo ageing changes and eventually come to stain as collagen [59, 60]. It is interesting to note that on the basis of its amino acid composition, Spiro [105] has suggested that the basement membrane belongs to the collagen family of proteins.

The thickening of the basement membrane that occurs in diabetes is not continuous along the entire capillary but is segmental in nature [53, 124]. If the excessive accumulations of basement membrane material were the consequence of a generalized disorder in production or removal, a more uniform thickening would be anticipated. The patchy and irregular thickening is more consistent with localized filtration and deposition. The relationship of the basement membrane thickening to the increased diameter of the intracellular cleft in diabetes [113] requires more study.

Regional variations in muscle capillary basement membrane thickness have been reported in normal subjects [92, 115, 125, 126]. Electron microscopic studies of diabetic capillaries have demonstrated that the basement membrane of most, if not all, tissues is thickened, although

perhaps not to the same extent [92, 125]. Electron microscopic examination of the capillary basement membrane in the glomerulus, retina and skeletal muscle of chronically streptozotocin-diabetic rats has shown that the basement membrane thickens at different rates in these tissues [12]. In addition to longitudinal permeability gradients [128], there are regional differences in capillary permeability [42, 64, 108]. It would be worth investigating the relationship between the regional differences in capillary permeability and the thickness of the capillary basement membrane. If the function of the capillary basement membrane is eventually defined, this information would be one of the important pieces of the puzzle.

As a result of the interaction between hydrostatic and colloid osmotic pressures, the amount of fluid leaving the microcirculation radial to the blood flow is of the order of 0.1% of the local blood flow [47]. In the glomerular capillary, the filtration fraction is of the order of 20% of the renal plasma flow. The glomerular capillary with the greatest filtration volume of any capillary in the body has a basement membrane 10 to 20 times thicker than that of any other capillary. In diabetes, not only does the basement membrane thicken, but the capillary permeability also increases. This suggests that the basement membrane may, in addition to its role as a molecular sieve, function as an osmotic imbiber of water and contribute to the tissue oncotic pressure in the Starling equation for transcapillary fluid exchange.

The physiologic role of the lymphatic system in maintaining interstitial fluid volume has become more appreciated in recent years [42, 43, 45, 47]. Pertubations of the Starling parameters that result

in an increased transcapillary filtration would cause an increase in the interstial fluid volume unless compensated for by adjustments in lymph flow. The increase in capillary permeability of diabetic patients suggests that lymphatic activity would have to be enhanced in order to maintain interstial fluid volume.

Without injection of a 1-2% solution of Evan's blue dye in physiological saline, the lymphatic terminations do not show up against the translucent ground substance of the mesentery [129]. Collecting lymphatics are prominent. They are distinquished by the presence of valve leaflets and show spontaneous contractions.

Lymphatics were rarely seen in the mesentery of non-diabetic animals. They were obscured from view by the heavy deposits of fat in the mesentery. The same decrease in fat cell size that exposed the complete distribution and branching pattern of the terminal microvasculature also exposed the mesenteric lymphatics in the diabetic animals.

Only collecting lymphatics were seen in this series of experiments. The contraction-relaxation cycles were most rapid in the larger collecting channels (150-250 microns). The periodicity of the contractions (7-33/min) cover a broader range than the 15-18 contractions per minute reported by Zweifach and Prather [129] for lymphatics of this size. The qualitative evidence accumulated in this study does not give any definitive answer concerning enhanced lymphatic activity in diabetic animals.

CHAPTER VI

SUMMARY

The microvascular manifestations of diabetes mellitus present a serious clinical problem for the diabetic patient, far more so than do the derangements in carbohydrate metabolism. Little is known about the basic pathogenesis of diabetic microangiopathy. Alterations in blood flow to various organs appear before pathological lesions. The purpose of this study was to qualitatively examine the effects of diabetes mellitus on circulatory dynamics within a microvascular network. This was achieved by measuring the red cell velocity in the mesenteric microcirculation of an animal model of diabetes mellitus.

The red cell velocity in the arterioles, capillaries and venules of the control animals was 0.60 ± 0.10 mm/sec, 0.45 ± 0.09 mm/sec and 0.34 ± 0.07 mm/sec, respectively. These results are comparable to those reported in the literature and with previous results obtained in this laboratory. The red cell velocity measured in the arterioles (0.68 ± 0.12 mm/sec), capillaries (0.24 ± 0.03 mm/sec) and venules (0.30 ± 0.04 mm/sec) of the short-term diabetic animals do not differ significantly from control values. The red cell velocity in all segments of the microcirculation of the long-term diabetic animals was greater than that measured in either the control or short-term diabetic animals. For the long-term diabetic animals the values for the red cell velocity in the arterioles, capillaries and venules were 1.22 ± 0.15 mm/sec, 0.59 ± 0.12 mm/sec and 0.70 ± 0.08 mm/sec, respectively.

The increase in red cell velocity in the long-term diabetic animals is not due to an increase in blood pressure or outside diameter of the blood vessels. The increased red cell velocity may be due to either an increase in the inside radius to wall thickness ratio, a decrease in the effective cell volume due to moderate rouleaux formation and/or increased plasma viscosity, or a change in the interaction of red cells with the endo-endothelial lining of blood vessels such that resistance to flow is lessened.

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APPENDIX A

METABOLIC INFORMATION

CONTROL ANIMALS

Preparation Number	Age of Rat (days)	Duration of Diabetes (days)	Weight (grams)	Plasma Glucose (mg/dl)
1	90	0	430	
2	85	0		53
4	81	0	300	73
7	82	0	330	99
10	95	0	350	57
11	97	0	400	
16	117	0	400	120
19	81	0	290	108
21	114	0	365	109
22	101	0	335	74
23	101	0	332	
31	93	0	320	74
32	97	0	330	60
35	98	0	350	102
37	110	0	410	60
N	15	15	14	12
Total	1442	0	4942	989
Mean	96.13	0	353	82.42
Standard Deviation	11.43	0	42.47	23.70
Variance	130.64	0	1803.70	561.69
S.E.M.	2.95	0	11.35	6.84

SHORT-TERM DIABETIC ANIMALS

Preparation Number	Age of Rat (days)	Duration of Diabetes (days)	Weight (grams)	Plasma Glucose (mg/dl)
3	85	3	325	183
5	81	7	310	211
6	82	8	310	170
12	97	7	300	
13	98	8	260	229
18	81	8	250	
20	82	9	227	430
24	82	7	300	240
25	97	7	270	
26	98	8	260	332
27	99	9	260	278
28	84	9	246	384
N	12	12	12	9
Total	1066	90	3318	2457
Mean	88.83	7.5	276.5	273
Standard Deviation	8.01	1.62	31.11	90.87
Variance	64.16	2.62	967.83	8257.36
S.E.M.	2.31	0.47	8.98	30.29

LONG-TERM DIABETIC ANIMALS

	Preparation Number	Age of Rat (days)	Duration of Diabetes (days)	Weight (grams)	Plasma Glucose (mg/dl)
	8	96	14	300	222
	9	95	21	240	147
	14	112	30	280	434
	15	117	35	280	409
	17	118	36	290	500
	29	90	14	250	269
	30	104	14	293	107
	33	97	21	230	292
	34	98	22	230	217
	36	113	23	280	419
	38	110	35	220	401
	39	112	37	300	346
N		12	12	12	12
Total		1262	302	3193	3556
Mean		105.17	25.17	266.08	296.33
Standard Deviation		9.63	9.03	29.92	125.19
Variance		92.74	81.54	895.21	15672.54
S.E.M.		2.78	2.61	8.64	36.14

APPENDIX B

DATA
Red Cell Velocity
Vessel Dimensions
Average Mean Systemic Pressure

ARTERIOLES: CONTROL ANIMALS

Preparation Number	Red Cell Velocity (mm/sec)	Vessel Diameter (microns)	Average Mean Systemic Pressure (mm/Hg)
4	0.401	15	156
	0.478	15	157
	0.649	12	147
	0.244	18	130
16	0.219	10	153
19	0.51	10	160
	0.42	10	145
21	0.30	10	170
	0.27	15	160
32	1.47	15	150
	0.55	10	150
	1.10	15	150
	0.83	10	115
	0.93	10	165
N	14	14	14
Mean	0.5979	12.5000	150.5710
Standard Deviation	0.3660	2.8488	14.0697
Variance	0.1340	8.1154	197.9560
S.E.M.	0.0978	0.7614	3.7603

ARTERIOLES: SHORT-TERM DIABETIC ANIMALS

Preparation Number	Red Cell Velocity (mm/sec)	Vessel Diameter (microns)	Average Mean Systemic Pressure (mm/Hg)
3	0.021	30	120
	0.049	25	107
	0.169	20	95
5	1.268	12	122
6	0.884	12	120
	1.794	15	130
	0.695	10	120
12	0.323	10	151
	0.514	9	127
	0.426	20	115
	0.264	30	107
13	0.893	25	135
	0.357	15	150
20	0.45	12	150
	0.79	10	150
	2.34	10	156
26	0.15	10	155
	0.30	10	148
27	0.43	14	145
	0.36	12	150
	1.39	22	147
28	1.07	12	142
	0.53	9	141
	0.86	11	145
N	24	24	24
Mean	0.6800	15.2083	134.5000
Standard Deviation	0.5664	6.6983	17.5774
Variance	0.3208	44.8677	308.2610
S.E.M.	0.1156	1.3673	3.5839

ARTERIOLES: LONG-TERM DIABETIC ANIMALS

Preparation Number	Red Cell Velocity (mm/sec)	Vessel Diameter (microns)	Average Mean Systemic Pressure (mm/Hg)
14	0.387	18	100
30	1.08	10	144
	0.64	9	144
	1.99	10	144
	0.80	9	144
	0.41	10	144
	0.52	9	128
33	2.66	10	144
	1.06	10	115
34	1.45	8	150
	2.45	15	148
	0.86	9	148
36	0.81	9	135
	1.57	18	135
	0.89	9	135
	1.74	18	139
	2.16	18	120
38	0.97	12	135
39	0.83	15	147
	1.12	15	148
N	20	20	20
Mean	1.2200	12.0500	137.3500
Standard Deviation	0.6702	3.7060	12.9097
Variance	0.4491	13.7342	166.6600
S.E.M.	0.1499	0.8287	2.8867

CAPILLARIES: CONTROL ANIMALS

Preparation Number	Red Cell Velocity (mm/sec)	Vessel Diameter (microns)	Average Mean Systemic Pressure (mm/Hg)
1	0.26	10	167
4	0.136	7	138
	0.084	7	113
7	0.574	8	120
	1.288	8	120
	0.708	5	95
	0.677	8	133
16	0.166	6	148
	0.293	7	153
	0.15	9	155
19	0.15	10	160
	0.66	8	160
	0.23	8	160
21	0.21	7	164
	0.23	7	164
32	0.59	11	158
	0.46	5	160
	1.21	11	155
N	18	18	18
Mean	0.4489	7.8889	145.7220
Standard Deviation	0.3589	1.7786	21.0120
Variance	0.1288	3.1634	441.5060
S.E.M.	0.0846	0.4192	4.9526

CAPILLARIES: SHORT-TERM DIABETIC ANIMALS

Preparation Number	Red Cell Velocity (mm/sec)	Vessel Diameter (microns)	Average Mean Systemic Pressure (mm/Hg)
3	0.091	10	117
6	0.239	6	112
	0.066	8	112
12	0.395	7	150
	0.380	6	151
	0.520	10	151
20	0.075	8	147
	0.25	9	157
	0.10	9	144
	0.129	8	140
	0.23	8	140
	0.27	8	150
	0.21	4	150
26	0.35	7	150
	0.16	8	140
	0.12	10	137
	0.24	8	130
27	0.10	5	150
	0.15	10	140
28	0.55	6	141
	0.27	8	144
	0.38	6	145
N	22	22	22
Mean	0.2404	7.6818	140.8180
Standard Deviation	0.1400	1.6729	12.5911
Variance	0.0196	2.7987	158.5360
S.E.M.	0.0299	0.3567	2.6844

CAPILLARIES: LONG-TERM DIABETIC ANIMALS

Preparation Number	Red Cell Velocity (mm/sec)	Vessel Diameter (microns)	Average Mean Systemic Pressure (mm/Hg)
14	0.344	8	135
	0.168	6	103
	0.104	10	130
	0.086	8	128
30	0.84	4	144
	0.53	10	144
	0.48	8	144
33	0.20	7	120
	0.37	5	115
34	1.75	5	140
	2.34	6	148
	0.42	5	146
36	0.47	10	133
	0.21	10	132
	0.26	10	137
	0.57	10	139
38	0.78	8	118
	0.94	8	142
39	0.53	9	123
	0.45	7	130
N	20	20	20
Mean	0.5920	7.7000	132.5500
Standard Deviation	0.5570	2.0026	11.9229
Variance	0.3102	4.0105	142.1550
S.E.M.	0.1245	0.4478	2.6660

VENULES: CONTROL ANIMALS

Preparation Number	Red Cell Velocity (mm/sec)	Vessel Diameter (microns)	Average Mean Systemic Pressure (mm/Hg)
1	0.075	15	164
	0.307	20	173
4	0.055	30	123
	0.727	30	138
	0.129	35	133
7	0.761	28	100
16	0.390	15	143
21	0.140	11	170
	0.190	20	170
32	0.49	20	150
	0.28	25	145
	0.49	25	145
N	12	12	12
Mean	0.3375	22.8333	146.1670
Standard Deviation	0.2399	7.2216	21.5526
Variance	0.0575	52.1515	464.5150
S.E.M.	0.0693	2.0847	6.2217

VENULES: SHORT-TERM DIABETIC ANIMALS

Preparation Number	Red Cell Velocity (mm/sec)	Vessel Diameter (microns)	Average Mean Systemic Pressure (mm/Hg)
3	0.122	40	90
6	0.031	25	135
	0.047	28	130
	0.267	9	120
	0.018	15	120
12	0.349	17	151
	0.413	11	127
13	0.599	35	140
20	0.60	10	155
	0.26	13	150
	0.45	15	150
	0.30	20	150
	0.38	12	150
26	0.11	12	148
	0.19	8	148
27	0.15	15	150
	0.23	14	150
	0.37	20	140
28	0.61	18	140
	0.45	14	137
	0.29	25	141
N	21	21	21
Mean	0.2971	17.9048	139.1430
Standard Deviation	0.1832	8.4552	15.2685
Variance	0.0336	71.4904	233.1280
S.E.M.	0.0400	1.8451	3.3319

VENULES: LONG-TERM DIABETIC ANIMALS

Preparation Number	Red Cell Velocity (mm/sec)	Vessel Diameter (microns)	Average Mean Systemic Pressure (mm/Hg)
14	0.124	30	103
30	0.69	20	144
	0.97	11	144
	0.69	15	140
	0.38	20	135
33	0.94	12	115
	0.53	10	118
	0.96	18	100
34	0.48	16	150
	1.37	10	148
36	0.26	25	135
	0.72	13	139
	0.49	13	129
	0.68	25	140
	0.48	25	111
38	0.72	12	122
	0.63	25	127
	1.48	10	135
	1.11	20	139
	1.08	13	140
39	0.43	28	152
	0.17	15	124
N	22	22	22
Mean	0.6991	17.5455	131.3640
Standard Deviation	0.3606	6.3899	14.7440
Variance	0.1300	40.8311	11.2238
S.E.M.	0.0769	1.3623	3.1434

APPENDIX C

STATISTICS

RED CELL VELOCITY

ARTERIOLES:

ANOVA

Source of Variation	df	Sum of Squares	Mean Squares	F
Between Groups	2	4.3209	2.1605	6.7310
Within Groups	55	17.6534	0.3210	
Total	57	21.9743		

SCHEFFE CONTRAST

	Short-term	Long-term
Control	0.19	9.93
Short-term		9.91

CAPILLARIES:

ANOVA

Source of Variation	df	Sum of Squares	Mean Squares	F
Between Groups	2	1.3163	0.6581	4.4156
Within Groups	57	8.4956	0.1490	
Total	59	9.8119		

SCHEFFE CONTRAST

	Short-term	Long-term
Control	2.89	1.30
Short-term		8.69

VENULES:

ANOVA

Source of Variation	df	Sum of Squares	Mean Squares	F
Between Groups	2	1.9922	0.9961	12.8386
Within Groups	52	4.0344	0.0776	
Total	54	6.0266		

SCHEFFE CONTRAST

	Short-term	Long-term
Control	0.16	13.08
Short-term		22.38

DIAMETER

ARTERIOLES:

ANOVA

Source of Variation	df	Sum of Squares	Mean Squares	F
Between Groups	2	126.0230	63.0117	2.4783
Within Groups	55	1398.4100		
Total	57	1524.4330		

SCHEFFE CONTRAST

	Short-term	Long-term
Control	2.55	0.08
Short-term		4.28

CAPILLARIES:

ANOVA

Source of Variation	df	Sum of Squares	Mean Squares	F
Between Groups	2	0.4990	0.2495	0.0753
Within Groups	57	188.7500	3.3114	
Total	59	189.2490		

SCHEFFE CONTRAST

	Short-term	Long-term
Control	0.13	0.11
Short-term		0.001

VENULES:

ANOVA

Source of Variation	df	Sum of Squares	Mean Squares	F
Between Groups	2	246.5980	123.2990	2.2411
Within Groups	52	2860.9300	55.0178	
Total	54	3107.5300		

SCHEFFE CONTRAST

	Short-term	Long-term
Control	3.71	1.09
Short-term		3.95

AVERAGE MEAN SYSTEMIC PRESSURE

ARTERIOLES:

ANOVA

Source of Variation	df	Sum of Squares	Mean Squares	F
Between Groups	2	2407.25	1203.63	5.1597
Within Groups	55	12830.00	233.272	
Total	57	15237.20		

SCHEFFE CONTRAST

	Short-term	Long-term
Control	9.79	6.17
Short-term		0.38

CAPILLARIES:

ANOVA

Source of Variation	df	Sum of Squares	Mean Squares	F
Between Groups	2	1701.25	850.625	3.5820
Within Groups	57	13535.80	237.471	
Total	59	15237.10		

SCHEFFE CONTRAST

	Short-term	Long-term
Control	1.06	6.92
Short-term		3.02

VENULES:

ANOVA

Source of Variation	df	Sum of Squares	Mean Squares	F
Between Groups	2	1786.25	893.125	3.2393
Within Groups	52	14337.30	275.718	
Total	54	16123.60		

SCHEFFE CONTRAST

	Short-term	Long-term
Control	1.37	6.17
Short-term		2.36

APPROVAL SHEET

The thesis submitted by Karl M. Nelson has been read and approved by the following committee:

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The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the Committee with reference to content and form.

January 7, 1977

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