Effect of streptozotocin-diabetes on the local and general responses to injury in the rat

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Summary

1. The effects of streptozotocin-diabetes on the local and general responses to a 4 h period of bilateral hind-limb ischaemia in the rat have been investigated. The rats were injured 48 h after the intravenous injection of the streptozotocin.

2. Less fluid was lost from the circulation into the injured limbs after injury in the diabetic rats and this was directly related to the severity of the diabetes, but could not be explained by dehydration. However, when the diabetic and non-diabetic injured rats were considered together there was a significant negative correlation between either plasma osmolality or plasma glucose concentration and water content in the injured hind limb.

3. The relationship between plasma glucose concentration and plasma osmolality was changed by injury such that, particularly in the injured diabetic rats, plasma osmolality at a given glucose concentration was higher than that predicted from the relationship between these variables in the uninjured rat.

Key words: diabetes mellitus, microcirculation, oedema, shock traumatic, streptozotocin.

Introduction

Trauma is known to have profound effects on carbohydrate metabolism. Hyperglycaemia occurs after injury in man and other animals with impairment of peripheral sensitivity to insulin or suppression of insulin secretion (Butterfield, 1955; Allison, Prowse & Chamberlain, 1967; Frayn, 1975). However, it is not clear whether a pre-existing diabetic state modifies, or is itself altered by, the metabolic responses to injury, although Ingle & Nezamis (1950) noted that injury produced by the subcutaneous injection of formaldehyde decreased the glycosuria of diabetes. The local response to injury may also be altered by diabetes mellitus, for there is increasing evidence that diabetes has marked effects on the inflammatory response (Goth, Nash, Nagler & Holman, 1957; Garcia Leme, Hamamura, Migliorini & Leite, 1973; Llorach, Böhm & Garcia Leme, 1976) and on wound healing (Herbsman, Powers, Hirschman & Schaftan, 1968; Abbey, Cohen & Shklar, 1972; Weringer, Sugiyama, Schug & Arquilla, 1975).

The aim of the present study was to investigate the interactions between a standard injury and experimental diabetes mellitus in the rat. A model of acute diabetes was chosen so that any interactions could be studied without the complication of the structural changes in the microcirculation characteristic of chronic diabetes (e.g. Starr, 1930; Handelsman, Levitt & Conrad, 1952; Greeson, Freedman, Levan & Wong, 1975). Diabetes was produced by the intravenous injection of streptozotocin, which selectively destroys the pancreatic β cells, the severity of the diabetes being dose-dependent (Rakieten, Rakieten & Nadkarni, 1963; Schein, Alberti & Williamson, 1971). It was expected, but not found, that diabetes would mainly affect the metabolic responses to severe...
injury. Our studies revealed that, in addition to the changes in fluid distribution produced by the diabetic state, the pattern of fluid redistribution after injury was also altered. Some of the results have been presented in preliminary communications (Little, Stoner & Elebute, 1977; Little & Elebute, 1977).

Methods

Male Porton-Wistar albino rats (weight range 220–265 g) fed on MRC diet 41B were kept from weaning in an ambient temperature of 18–22°C with 12 h light per day from 07.00 to 19.00 hours. Diabetes was produced by the intravenous injection of 75 or 150 mg of streptozotocin (Upjohn Co., Kalamazoo, Michigan, U.S.A.)/kg body weight immediately after solution in disodium citrate buffer (0·01 mol/l), pH 4·5. The rats were given 5% (w/v) glucose solution to drink during the next 24 h. At 48 h after injection and immediately before injury the urine was tested for ketone bodies and glucose with reagent strips (Keto-Diastix, Miles Laboratories Ltd, Slough, U.K.).

The injury was a 4 h period of bilateral hind-limb ischaemia produced with rubber tourniquets applied during a short period (approximately 3 min) of ether anaesthesia by Rosenthal’s (1943) method. Dehydration was produced in non-diabetic rats by the intraperitoneal implantation, under ether anaesthesia, of Lyphogel (Gelman-Hawksley Ltd, Lancing, Sussex, U.K.), a polyacrylamide hydrogel which expands in aqueous solutions to absorb exactly five times its own weight of water and low-molecular-weight solutes in 5 h while excluding proteins and other substances with a molecular weight of 20 000 or more. The properties of the crystals are the same in vivo and in vitro (Little, 1971). As 95% of the fluid uptake by the crystals occurs within 4 h the crystals were implanted when the tourniquets were applied.

The water content of the hind limbs and the actual fluid loss from the circulation (expressed as ml/100 g body weight) after bilateral hind-limb ischaemia was calculated as described by Little (1972). The apparent fluid loss from the circulation was calculated from the changes in great vessel packed cell volume corrected for $F_{cells}$, that is the ratio of whole-body packed cell volume to great-vessel packed cell volume (Gregersen & Rawson, 1959) and the measured erythrocyte volume (Little, 1974). The great vessel packed cell volume was obtained either from arterial blood taken from a PE 10 (Clay-Adams, Intramedic) cannula in the ventral caudal artery or from mixed arterial/venous blood collected by decapitation into a heparinized beaker. The blood was taken into heparinized microhaematocrit tubes and spun for 1 h at 4000 rev./min in an MSE centrifuge. A factor of 3% was used to correct for trapped plasma (Constable, 1963). Plasma volumes and erythrocyte volumes were measured simultaneously with $^{125}$I-labelled human serum albumin and $^{51}$Cr-labelled erythrocytes as described by Cunningham (1975). $F_{cells}$ was calculated from the simultaneously measured erythrocyte and plasma volumes and the measured great-vessel packed cell volume.

Plasma glucose concentrations were measured with the hexokinase assay kit (Boehringer Corporation, London, Ltd). Plasma osmolality was measured with a model 3D Advanced Osmometer (Advanced Instruments Inc., Needham Heights, Mass., U.S.A.). Colon temperature was measured with a thermocouple (Elektrolaboratoriet, Copenhagen) 6–8 cm from the anus.

Where possible the results have been expressed as mean value ± SE and the mean values have been compared by the Student’s $t$-test as modified by Fisher (1934) for small samples.

Results

Control observations of the diabetic state

The body weight of the rats decreased by 11·5 ± 0·6 g/100 g body weight ($n = 31$) during the 48 h after streptozotocin (75 mg/kg) and by 18·4 ± 0·7 g/100 g body weight ($n = 26$) after the 150 mg/kg dose. During the same period the body weight of non-diabetic control rats increased by 2·7 ± 0·4 g/100 g body weight ($n = 10$). At 48 h after the injection of streptozotocin there were dose-related increases in plasma glucose concentration and in plasma osmolality (Table 1), measured in the post-absorptive state (Heath & Threlfall, 1968).

Glycosuria was seen in all the streptozotocin-treated rats; ketonuria was observed in all rats given streptozotocin (150 mg/kg) but in only 60% of those given the 75 mg/kg dose.

Because of the rapid fall in the body weight of the diabetic rats the measured plasma and erythrocyte volumes have been related to the body weight at the time of streptozotocin injection. The measured plasma and erythrocyte volumes of the control rats were the same as those found previously in the same rat colony (Heath, 1973). The measured plasma volume was lower in the streptozotocin-treated rats (Table 1) and as further
TABLE 1. Effect of streptozotocin injection on plasma glucose concentration, plasma osmolality, measured plasma and erythrocyte volumes and $F_{\text{cell}}$ in the post-absorptive rat

Results are expressed as mean ± se. The numbers of animals are shown in parentheses. Significance of differences from corresponding control values: $^*P < 0.05$; $^{**}P < 0.02$; $^{***}P < 0.01$; $^{****}P < 0.001$.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Streptozotocin 75 mg/kg</th>
<th>Streptozotocin 150 mg/kg</th>
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<tbody>
<tr>
<td>Plasma glucose (mmol/l)</td>
<td>8.3 ± 0.2 (6)</td>
<td>22.7 ± 1.6 (6)$^{****}$</td>
<td>31.8 ± 2.3 (6)$^{****}$</td>
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<tr>
<td>Plasma osmolality (mosmol/kg of water)</td>
<td>300.3 ± 4.1 (6)</td>
<td>310.2 ± 1.7 (5)</td>
<td>328.8 ± 4.5 (6)$^{***}$</td>
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<tr>
<td>Plasma volume (ml/100 g body wt.)†</td>
<td>3.92 ± 0.07 (11)</td>
<td>3.21 ± 0.15 (7)$^{***}$</td>
<td>2.77 ± 0.12 (12)$^{****}$</td>
</tr>
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<td>Erythrocyte volume (ml/100 g body wt.)†</td>
<td>2.21 ± 0.03 (11)</td>
<td>2.43 ± 0.08 (7)$^{*}$</td>
<td>2.11 ± 0.06 (12)</td>
</tr>
<tr>
<td>$F_{\text{cell}}$†</td>
<td>0.824 ± 0.005</td>
<td>0.851 ± 0.006$^{**}$</td>
<td>0.850 ± 0.008$^{*}$</td>
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† In streptozotocin-treated animals, volumes related to pre-injection weight.

‡ Ratio of whole-body packed cell volume to great-vessel packed cell volume (Gregersen & Rawson, 1959).

TABLE 2. Effect of streptozotocin-diabetes and Lyphogel pretreatment on the 'actual' and 'apparent' fluid loss from the plasma and on the changes in hind-limb water content, plasma osmolality and plasma glucose concentration after 4 h of bilateral hind-limb ischaemia (10.00 to 14.00 hours) in the rat

Results are expressed as mean ± se. The numbers of animals are shown in parentheses. Significance of differences from control value: $^*P < 0.05$; $^{**}P < 0.02$; $^{***}P < 0.01$; $^{****}P < 0.001$. Significance of differences from corresponding 'actual' value: $\dagger P < 0.05$; $\dagger\dagger P < 0.02$; $\dagger\dagger\dagger P < 0.01$. Significance of differences from corresponding value at time of tourniquet release: at $\ddagger P < 0.05$; $\ddagger\ddagger P < 0.01$; $\ddagger\ddagger\ddagger P < 0.001$. Significance of difference from corresponding 'uninjured' value: $§ P < 0.02$; $§§ P < 0.01$.

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<th></th>
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<th>Lyphogel</th>
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<tr>
<td>Hind-limb water content (g/dry wt.)</td>
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<tr>
<td>(i) Uninjured</td>
<td>2.12 ± 0.04 (5)</td>
<td>2.00 ± 0.04 (5)</td>
<td>1.82 ± 0.07 (5)</td>
<td>—</td>
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<tr>
<td>(ii) At time of tourniquet release</td>
<td>2.06 ± 0.03 (6)</td>
<td>1.92 ± 0.03 (10)</td>
<td>1.87 ± 0.03 (11)</td>
<td>2.14 ± 0.03 (6)</td>
</tr>
<tr>
<td>(iii) 1 h after release</td>
<td>2.92 ± 0.03 (6)$^{***}$</td>
<td>2.44 ± 0.05 (6)$^{***}$</td>
<td>2.09 ± 0.06 (13)$^{***}$</td>
<td>2.76 ± 0.04 (12)$^{***}$</td>
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<tr>
<td>(iv) 3 h after release</td>
<td>3.21 ± 0.10 (6)</td>
<td>2.34 ± 0.09 (6)</td>
<td>2.17 ± 0.07 (6)</td>
<td>—</td>
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<tr>
<td>(v) Increase (g) during first hour</td>
<td>0.86 ± 0.04</td>
<td>0.52 ± 0.06$^{***}$</td>
<td>0.22 ± 0.07$^{****}$</td>
<td>0.62 ± 0.05$^{**}$</td>
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Fluid loss from plasma (ml/100 g body wt.)

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<tr>
<th></th>
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<th>Streptozotocin 150 mg/kg</th>
<th>Lyphogel</th>
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<tbody>
<tr>
<td>(i) Actual</td>
<td>2.26 ± 0.21 (5)</td>
<td>1.44 ± 0.20 (9)$^{*}$</td>
<td>1.74 ± 0.16 (5)</td>
<td>—</td>
</tr>
<tr>
<td>(ii) Apparent</td>
<td>1.35 ± 0.18 (5)$^{**}$</td>
<td>0.39 ± 0.09 (9)$^{***}$</td>
<td>0.97 ± 0.08 (5)$^{**}$</td>
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Volume added to circulation (i)–(ii)

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<tr>
<th></th>
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<th>Lyphogel</th>
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<tbody>
<tr>
<td>(iii) ml/100 g body wt.</td>
<td>0.91 ± 0.27</td>
<td>1.05 ± 0.21</td>
<td>—</td>
<td>0.78 ± 0.18</td>
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<tr>
<td>(iv) actual loss (%)</td>
<td>40.2 ± 6.4</td>
<td>65.4 ± 8.3</td>
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<td>42.3 ± 7.8</td>
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Plasma glucose (mmol/l)

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<tr>
<td>(i) Uninjured†</td>
<td>8.3 ± 0.2 (6)</td>
<td>22.7 ± 1.6 (6)</td>
<td>31.8 ± 2.3 (6)</td>
<td>—</td>
</tr>
<tr>
<td>(ii) At time of tourniquet release</td>
<td>10.2 ± 0.2 (6)$^{**}$</td>
<td>22.4 ± 1.0 (6)</td>
<td>29.8 ± 3.2 (11)$^{*}$</td>
<td>—</td>
</tr>
<tr>
<td>(iii) 1 h after release</td>
<td>15.7 ± 1.1 (6)$^{**}$</td>
<td>29.8 ± 0.8 (14)</td>
<td>33.2 ± 2.6 (12)</td>
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Plasma osmolality (mosmol/kg of water)

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<tr>
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<th>Lyphogel</th>
</tr>
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<tbody>
<tr>
<td>(i) At time of tourniquet release</td>
<td>276.3 ± 6.8 (6)</td>
<td>316.1 ± 8.4 (9)</td>
<td>350.7 ± 7.4 (11)</td>
<td>312.0 ± 5.4 (6)</td>
</tr>
<tr>
<td>(ii) 1 h after release</td>
<td>298.0 ± 3.0 (6)$^{*}$</td>
<td>327.1 ± 8.9 (10)</td>
<td>371.0 ± 9.3 (12)</td>
<td>319.7 ± 4.1 (6)</td>
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<tr>
<td>(iii) 3 h after release</td>
<td>312.7 ± 2.7 (6)$^{*}$</td>
<td>352.7 ± 7.9 (6)$^{*}$</td>
<td>378.4 ± 6.9 (5)</td>
<td>—</td>
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</table>
Time after application of tourniquets

FIG. 1. Changes in colon temperature of streptozotocin-diabetic (□, ■, 75 mg/kg; Δ, ▲, 150 mg/kg) and non-diabetic rats (○) during and after 4 h of bilateral hind-limb ischaemia at an environmental temperature of 20°C. The tourniquets were removed at the arrow. Mean values + SE are shown, with the number of animals in parentheses. Mean values significantly (P < 0.001) lower than the corresponding initial values are indicated by filled-in symbols (▲, ■, ●).

Evidence of dehydration the water content of the uninjured hind limbs was significantly reduced in the 150 mg/kg streptozotocin-treated rats (P < 0.05; Table 2). The measured erythrocyte volume was increased 48 h after streptozotocin at 75 mg/kg but unaltered by the higher dose. $F_{\text{cell}}$, the ratio of the calculated whole-body packed cell volume to the measured great-vessel packed cell volume, was higher in the streptozotocin-treated animals (Table 1).

Local response to limb ischaemia

During the 4 h bilateral hind-limb ischaemia the water content of the limbs was unchanged but within 60 min after removal of the tourniquets the water content of the injured limbs rose in all animals, although the increase was significantly less in the diabetic rats (Table 2). (Streptozotocin: 75 mg/kg, $P < 0.01$; 150 mg/kg, $P < 0.001$.) In non-diabetic rats the water content rose further during the next 2 h ($P < 0.05$) but this did not occur in the diabetic ones. A dose of Lyphogel was given to non-diabetic rats to increase great-vessel packed cell volume and plasma osmolality to the values found in the 75 mg/kg streptozotocin-treated rats immediately before tourniquet removal at the end of 4 h bilateral hind-limb ischaemia. Such dehydration decreased fluid loss after limb ischaemia but not as much as diabetes (Table 2).

General response to limb ischaemia

In the non-diabetic rats colon temperature was constant while the tourniquets were in place. In both groups of streptozotocin-diabetic rats colon temperature fell significantly during this period ($P < 0.001$), this being most marked in the 150 mg/kg group (Fig. 1). After removal of the tourniquets colon temperature fell in all three groups, but most rapidly in the non-diabetic rats.

After an initial rapid fall as the tourniquets were removed, arterial blood pressure remained constant for at least the next hour. The mean pressure during the first hour after 4 h bilateral hind-limb ischaemia in the 75 mg/kg streptozotocin-treated rats (110.2 ± 6.6 mmHg, $n = 5$) was higher ($P < 0.02$) than in the non-diabetic rats (82.5 ± 1.7 mmHg, $n = 12$) at the same time. The arterial pressure during the same period in the 150 mg/kg streptozotocin-treated rats (67.6 ± 8.6 mmHg, $n = 5$) was lower ($P < 0.02$) than in the 75 mg/kg streptozotocin-treated rats but not significantly different from that in the non-diabetic rats.

The decrease in plasma volume after the 4 h period of ischaemia was calculated from the great-vessel packed cell volume 1 h after tourniquet removal. The decrease in plasma volume can be considered as the 'apparent' fluid loss from the circulation, whereas, as described above, the 'actual' fluid loss from the circulation was given by the increase in water content of the injured limbs. The 'apparent' and the 'actual' fluid losses were less in the 75 mg/kg streptozotocin-treated rats than in either the non-diabetic or the Lyphogel-treated rats (Table 2). The 'apparent' fluid loss was less than the 'actual' fluid loss in all three groups. The difference between the 'actual' and the 'apparent' fluid loss from the circulation into the injured limbs, i.e. the volume of fluid passing into the circulation (compensation) from the uninjured tissues, was not affected by pretreatment with either streptozotocin or Lyphogel (Table 2).

Plasma glucose concentration rose in the non-diabetic rats while the tourniquets were in place. This increase was not seen in the already hyperglycaemic streptozotocin-diabetic rats (Table 2). In the first hour after tourniquet removal plasma glucose concentration increased in both the non-
Diabetes and injury in the rat

Diabetic and 75 mg/kg streptozotocin-diabetic rats, but not the 150 mg/kg streptozotocin-diabetic rats.

In the non-diabetic rats plasma osmolality rose during the 3 h period after removal of the tourniquets (Table 2), but did not increase during the first hour after tourniquet removal in either the streptozotocin- (75 mg/kg) or the Lyphogel-pretreated rats, although it was higher at 3 h after tourniquet removal in the 75 mg/kg streptozotocin-treated rats. There was no significant change in plasma osmolality after 4 h of limb ischaemia in the 150 mg/kg streptozotocin-treated rats.

When the streptozotocin-diabetic and non-diabetic uninjured rats were considered as one group there was a positive correlation \((P < 0.001)\) between plasma osmolality and plasma glucose concentration. The equation of the regression line was:

\[
\text{Plasma osmolality (mosmol/kg of water)} = (1.11 \pm 0.23)x + 290.3 \pm 5.3
\]

(where \(x\) is the plasma glucose concentration in mmol/l).

In the injured diabetic and non-diabetic rats this relationship was changed. At the end of 4 h bilateral hind-limb ischaemia and 1 h later significant numbers of the data points \((X^2\) test) were outside the 95% confidence limits of the 'uninjured' regression line \((15 \text{ out of 25}, P < 0.001\) and 15 out of 26, \(P < 0.001\) respectively). Of these nine were above the limits \((P < 0.001)\) at the end of the period of ischaemia and 13 were above the limits \((P < 0.001)\) 1 h later. A separate regression line could be calculated for the injured rats. The equation of the regression line was:

\[
\text{Plasma osmolality (mosmol/kg of water)} = (3.00 \pm 0.36)x + 255.1 \pm 9.7
\]

\[(r = 0.77, P < 0.001)\]

(where \(x\) is the plasma glucose concentration in mmol/l). The slope of this line was steeper than that in the uninjured rats \((P < 0.001)\).

When the data from the non-diabetic and streptozotocin-diabetic rats at 1 h after the end of the 4 h bilateral hind-limb ischaemia were grouped together there were significant \((P < 0.001)\) negative correlations between either plasma osmolality or plasma glucose concentration and hind-limb water content. The equations of the regression lines were as follows:

\[
\text{Hind-limb water content (g/g dry wt.)} = (-0.033 \pm 0.005)x + 3.337 \pm 0.139
\]

or

\[
= (-0.0086 \pm 0.0008)y + 5.331 \pm 0.265
\]

(where \(x\) is the plasma glucose concentration in mmol/l and \(y\) is the plasma osmolality in mosmol/kg of water).

Discussion

Diabetes mellitus was expected to influence the response to injury through effects on intermediary metabolism, but there were also alterations in the pattern of fluid redistribution after injury. The streptozotocin-induced diabetes was characterized by increases in plasma glucose concentration and osmolality, and by reduced plasma volume and tissue water content, which were all directly related to the dose of streptozotocin.

Ischaemic limb injury in the rat increases the blood concentration of glucose in two ways. First there is conversion of the lactate liberated from uninjured muscle by adrenaline into glucose in the liver, and secondly there is breakdown of liver glycogen after the period of limb ischaemia \((\text{Stoner \& Threlfall, 1960})\). Diabetic rats studied 48 h after the injection of streptozotocin did not show the rise during the period of ischaemia, and only those with the less-severe diabetes showed a raised plasma glucose after release of the tourniquets. This diminished response in diabetic rats may be due to depletion of muscle and liver glycogen in diabetes \((\text{von Mering \& Minkowski, 1890; Schein \textit{et al.}, 1971})\).

The increase in the ratio of the whole-body packed cell volume to the great-vessel packed cell volume \((F_{\text{cell}})\) in the diabetic rats was unexpected as it is unaffected by a variety of pathological conditions \((\text{Gregersen \& Rawson, 1959; Heath, 1973})\). However, a possible explanation is as follows. A 6-8 μm diameter erythrocyte must be readily deformed if it is to pass through a 4 μm diameter capillary \((\text{Braasch, 1971})\). Plasma osmolality was significantly increased in the streptozotocin-diabetic rats, and in a hyperosmotic medium erythrocytes are less easily deformed, thereby impeding the movement of erythrocytes through the capillaries. This will increase the mean transit time of the erythrocytes through the microcirculation \((\text{Effros, 1972})\) and increase the peripheral packed cell volume, relative to the great-vessel packed cell volume.

The streptozotocin-diabetic rats lost less fluid after injury, particularly in the severely diabetic rats \((150 \text{ mg of streptozotocin/kg})\), although these animals were in a poor condition. In addition to the loss of 20% of their pre-diabetic body weight, mean arterial blood pressure and body temperature were...
lower during the first hour after tourniquet removal than in the non-diabetic injured rats. Cooling and lowering of the arterial pressure both decrease the inflammatory response (Miles & Miles, 1952). Cruickshank (1954) attributed the poor inflammatory response in the alloxan-diabetic rabbit to circulatory failure. This cannot, however, explain the reduced post-ischaemic fluid loss in the 75 mg/kg streptozotocin-diabetic rats, as the mean arterial pressure during the first hour after 4 h bilateral hind-limb ischaemia in these rats was significantly higher than in the non-diabetic rats, yet the diabetic rats still lost significantly less fluid into the injured limbs.

The pre-ischaemic dehydration of the diabetic animals may be responsible for the reduction in post-ischaemic fluid loss, for similar reduction in plasma volume in the non-diabetic rats did not reduce the post-ischaemic fluid loss as much as in the diabetic rats. However, the Lyphogel pre-treatment did not produce the reduction in tissue water content found in the streptozotocin-treated rats.

These factors do not entirely explain why the streptozotocin-diabetic rats lost less fluid after injury. Other possible explanations may be related to the changes in plasma osmolality found in diabetes and after injury. Osmolality of tissue fluid and plasma are major factors controlling fluid movement across the endothelium of the exchange vessels (Lundvall, 1972; Mellander, 1974; Jarhult, 1975); at least 50% of the compensatory movement of fluid into the circulation after haemorrhage in the cat being due to hyperosmolality of plasma compared with extravascular fluid (Jarhult, 1975), the hyperosmolality can be almost completely accounted for by the hyperglycaemia found shortly after haemorrhage.

In uninjured diabetic and non-diabetic rats the relationship between plasma glucose concentration and plasma osmolality suggests that the hyperosmolality of the diabetic rats can be fully explained by their higher plasma glucose concentrations. However, in the injured rats the plasma osmolality, for a given glucose concentration, was higher than that predicted from the relationship in the uninjured rat, suggesting that, in the diabetic rat, injury increases the plasma amounts of some other osmotically active substance, as yet unidentified.

An increase in plasma osmolality relative to tissue fluid osmolality will have two effects in the injured rat. First, in the injured hind limbs it may limit the volume of fluid moving out of the circulation, as suggested by the inverse relationship between plasma osmolality and hind-limb water content. However, plasma osmolality will only influence fluid movement across the walls of the exchange vessels if their endothelium remains selectively permeable. The oedema fluid formed after a 4 h period of ischaemia has a high protein content (Little, 1972), suggesting that the permeability of the microcirculation to protein is increased in the injured tissues, but this may only be in the venules, the capillaries retaining their selective permeability. This is the typical pattern of response elicited by such mediators of an inflammatory response as histamine and serotonin (Majno, Palade & Schoefl, 1961).

In the uninjured tissues the permeability of the microcirculation is unaltered (Tabor, Rosenthal & Millican, 1951) and the post-ischaemic increase in plasma osmolality may help to increase the compensatory movement of fluid into the circulation. Such compensation, expressed as a percentage of the actual fluid loss, was not significantly greater in the diabetic rats. The ability to compensate for circulatory hypovolaemia during losses of 33% of blood volume was unaffected by experimental diabetes mellitus in the rat (Little et al., 1977).

Acknowledgments

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