Effects of Experimental Diabetes and Genetic Obesity on Regional Blood Flow in the Rat

Paul D. Lucas, Ph.D., and John M. Foy, Ph.D., Bradford, U. K.*

SUMMARY

Blood flow through various tissues of streptozotocin- and alloxan-diabetic and genetically obese rats was compared with that of controls by a radioisotopically labeled microsphere technique. Total cardiac output per unit body weight was unchanged in the diabetic groups but decreased in the obese animals. The proportion of cardiac output received by the kidney and organs of the gastrointestinal tract was increased in the diabetic animals. Tissue hyperplasia appeared to be largely responsible. Blood flow per unit weight was markedly increased in the fat tissue of diabetic rats but was reduced in that of the obese rats, indicating a positive relationship between fat mobilization and blood flow. Blood flow in the hindlimbs, tail, skin, and spleen were all reduced in at least one diabetic group. Most of the changes observed appeared to progress with the duration of diabetes. Possible hormonal and metabolic causes are discussed. Some of the experimental changes observed may form useful models for diabetic vasculopathy. DIABETES *26*:786-92, August, 1977.

The development of cardiovascular abnormalities in the human diabetic is now known to be accompanied by changes in regional blood flow.[1] Reversible increases in the blood flow of many tissues have been demonstrated in early diabetes. These include the forearm,[2] fat,[3,4] and retina.[5] In the kidney, blood flow is not markedly changed but glomerular filtration is increased.[6] In longer-term diabetics, retinal blood flow,[5] maximal flow in the extremities,[7,8] and glomerular filtration rate [9] tend to decline with the development of vasculopathy in these areas.

The above changes may be a factor in the rate of progression of diabetic vasculopathy. Reduction of blood flow to the kidneys (which may reduce glomerular filtration) by partial arterial occlusion may prevent the development of nephropathy.[10] Occlusion of the ophthalmic arteries may accelerate retinopathy.[11] The purpose of the present study was to determine whether changes in regional blood flow similar to those occurring in the human diabetic occur in the diabetic rat. If so, their pathologic effects, if any, may be more thoroughly investigated than is possible in the human diabetic. Prior to the development of the radioisotopically labeled plastic-sphere technique for the experimental determination of blood flow in particular organs, such studies were relatively difficult to perform. (In particular, detailed blood flow determinations on groups of experimentally diabetic animals have not been reported.) The sphere technique offers a convenient and previously validated method for studying blood flow in several organs simultaneously.[12]

A recent study in pithed diabetic rats[13] suggested that there may be a redistribution of blood flow away from the hindquarters in favor of other areas, particularly under conditions in which sympathetic tone is high. This could be largely responsible for the increased incidence of tail gangrene that has been observed in cold-stressed diabetic rats.[14] A diabetes-induced redistribution of blood flow away from the hindquarters in the experimental model would be of interest since maximal blood flow in the extremities of long-term diabetic patients has been reported as being reduced.[7,8]

Genetically obese rats were included in the present study because some of their metabolic abnormalities, such as raised plasma lipids and insulin insensitivity,[15] have been linked with vascular deterioration in human diabetes.[16,17]

MATERIALS AND METHODS

Measurement of Regional Blood Flow with Microspheres

Test animals were anesthetized with 60 mg./kg. pentobarbitone administered intraperitoneally. The right carotid artery was cannulated with poly-

From the School of Studies in Pharmacology, University of Bradford, Bradford, BD7 1DP, U.K.

*Present address: University of Al-Fateh, Tripoli, Libyan Arab Republic.

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propylene tubing (Portex pp25) connected to a pressure transducer via a three-way tap. This cannula was pushed slowly down the carotid until it entered the left ventricle of the heart. It was then secured in position. Entry into the ventricle was detected by the change from arterial to ventricular pressure recording. The right femoral artery was cannulated with similar tubing connected to a slow-withdrawal pump, the latter being adjusted to withdraw 0.67 ml./min. The pump was started and, providing withdrawal was constant, approximately 2×10^5 15-μ-diameter microspheres (Pharmacia) labeled with scandium-46 contained in 0.1 ml. of the recommended Ficoll 70 solution were injected via the ventricular cannula. The radioactivity of the syringe used for microsphere injection was measured before and after injection in order to determine the total activity injected (T). A crystal scintillation counter was used for all determinations of radioactivity.

Blood withdrawal from the femoral artery was continued for 30 seconds after the microsphere injection. The blood withdrawn was then flushed into a counting vial and its activity (W) measured. Cardiac output (CO) was determined as follows:

$$CO = \frac{T}{W} \times 0.67 \text{ ml./min.}$$

After removing a 2-ml. sample of blood from the inferior vena cava for glucose and free fatty acid estimations the tissues whose blood flows were to be determined were removed and weighed and their radioactivities measured. Their blood flows were then determined as follows:

$$\% \text{ CO to organ} = \frac{\text{Organ count}}{T} \times 100$$

$$\text{Blood flow (ml./100 gm./min.)} = \% \text{ CO} \times \text{CO (ml.)} \times \frac{\text{organ wt.}}{100 \text{ gm.}}$$

Blood flow in only the left hindleg was estimated since the right femoral artery had been obstructed by the blood-withdrawal cannula. Brain blood flow was probably reduced by the presence of the right carotid cannula. The nature of the brain vascular system, however, would result in reduction of blood flow to both sides of the brain. This experimental error, therefore, cannot be avoided by presenting data from only the left side of the brain.

A potential source of error in blood flow measurement by the procedure described above arises from alterations in blood volume. The effect of blood withdrawal from the arterial cannula, however, was to some extent compensated for by the injection into the left ventricle of a similar volume of the microsphere-containing fluid. Because of this it was considered that during the critical period between the start of blood withdrawal and the enlodgement of the microspheres in the vascular beds, blood volume alterations and, more particularly, physiologic responses to them would be minimal.

Alloxan and Streptozotocin Diabetes

Male CFE rats (200-300 gm.) received 1 ml./kg. of a freshly prepared solution of alloxan (50 mg./ml. saline), streptozotocin (60 mg./ml., pH 4.5, citrate buffer), or saline (0.9 per cent w/v NaCl solution) via a tail vein. The last group served as controls. Blood flow estimations were carried out on control, 3-, 14-, and 60-day streptozotocin-diabetic, and seven-day alloxan-diabetic rats. At no time were the animals denied food or water.

Obese Rats

Zucker obese rats were used at two to three months of age, by which time their metabolic abnormalities should be present.[14] Their nonobese littermates served as controls.

Blood Glucose and Plasma Free Fatty Acid Determination

Blood for both these determinations was withdrawn from the inferior vena cava about one minute after microsphere injection. Blood glucose was determined in 0.1-ml. samples of whole blood by a microcolorimetric copper-reduction method.[18] Plasma free fatty acids were estimated in 0.2-ml. samples of plasma by a colorimetric method.[19]

RESULTS

Effects of Diabetes

Diabetes of moderate severity was shown to occur in all groups treated with the diabetogens. Hyperglycemia was accompanied by normal or, in the case of the three-day streptozotocin-diabetic group, slightly raised blood free fatty acid levels (table 1).

Cardiac output (CO) was found to be very similar in control and diabetic groups (figure 1). Figure 1 also shows that the percentages of microspheres found in the lungs did not differ significantly between groups. These percentages were small and can largely be accounted for by direct blood supply to the respiratory tree. Microsphere recirculation was thus shown to be minimal.

Changes in per cent CO received by, blood flow (ml./100 gm./min.) in, and per cent body weight of various organs and tissues from control and diabetic groups are shown in tables 2, 3, and 4, respectively. The per cent CO received by the stomach, small intestine, and large intestine were all greater than controls' in at least two of the diabetic groups (table 2). These

TABLE 1

Blood glucose and free fatty acid (FFA) levels of the groups of rats used in the blood flow studies. Results are presented as mean ± standard error of the mean; n = number per group. SDR = streptozotocin-diabetic rats (3, 14, and 60 days after 60 mg./kg.), ADR = alloxan-diabetic rat (seven days after 50 mg./kg.) and LM = nonobese littermates of the obese rats. Significance of differences from controls indicated by * P<0.05 and † P<0.001.

Group	n	Blood glucose (mg./100 ml.)	Blood FFA (mM)
Controls	13	109 ± 6	0.651 ± 0.108
SDR 3	8	444 ± 21†	1.04 ± 0.11 *
14	10	384 ± 36†	0.632 ± 0.050
60	5	410 ± 44†	0.718 ± 0.092
ADR 7	7	562 ± 28†	0.822 ± 0.169
Obese	8	149 ± 16	2.76 ± 0.71*
L M	7	117 ± 11	0.987 ± 0.273

increases were accompanied by increases in the per cent body weight comprised by these organs (table 4). Increases in blood flow in the stomach and small intestine were, therefore, less marked, and no such increase was observed in the large intestine (table 3). The per cent CO received by the liver and kidneys was greater than control values in at least one of the diabetic groups (table 2). However, since the per cent body weight comprised by these organs was also increased (table 4), blood flows were not significantly changed (table 3).

The per cent CO received by the spleen, hindleg, hindpaw, and tail were all reduced in at least two diabetic groups (table 2). The tails of the shortest-term diabetic groups, however, received a greater per

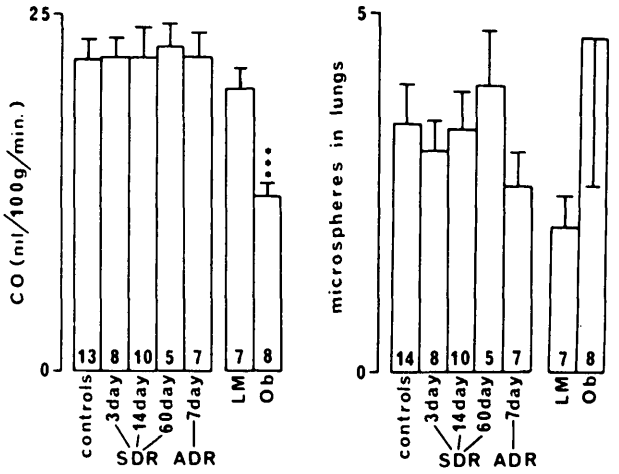

FIG. 1. These histograms represent the mean cardiac output (CO) per unit body weight and the mean percentage of microspheres found in the lungs of all groups used in the study. SDR = streptozotocin-diabetic rats, ADR = alloxan-diabetic rats, LM = nonobese littermates of the obese rats, and Ob = obese rats. Numbers per group are indicated at the base of each column.

cent CO than did those of control rats. The percentage of body weight contributed by the spleen was reduced in all diabetic groups (table 4). In spite of this, splenic blood flow was reduced in the two longest-term diabetic groups (table 3).

Adrenal blood flow was decreased in the longest-term diabetic group (table 3). The weight of these organs, however, was increased in this group (table 4), and their share of CO was consequently unchanged.

TABLE 2

The percentages of cardiac output received by various organs on control and diabetic rats (mean ± S.E.M.). Numbers per group are indicated in parentheses at the head of each column. N.D. = not done.

	Controls (15)	Streptozotocin-diabetic			Alloxan-diabetic
Organ		3-day (8)	14-day (10)	60-day (5)	7-day (7)
Stomach	1.7 ± 0.1	1.5 ± 0.2	3.0 ± 0.4 P<0.01	3.4 ± 0.6	2.0 ± 0.2
S. Intestine	18 ± 1	24 ± 2 P<0.01	26 ± 2 P<0.01	34 ± 3 P<0.001	32 ± 2 P<0.001
L. Intestine	5.8 ± 0.6	7.2 ± 0.6	6.9 ± 0.7	13 ± 2 P<0.001	8.3 ± 0.7 P<0.01
Kidneys	20 ± 1	26 ± 3 P<0.05	26 ± 3 P<0.05	21 ± 2	19.8 ± 1
Liver	3.9 ± 0.3	4.3 ± 0.7	5.6 ± 0.4 P<0.01	3.0 ± 0.5	4.5 ± 0.9
Spleen	2.4 ± 0.3	1.6 ± 0.3 P<0.05	0.80 ± 0.21 P<0.001	0.56 ± 0.17 P<0.001	1.3 ± 0.1 P<0.01
Adrenals	0.58 ± 0.05	0.69 ± 0.06	N.D.	0.68 ± 0.08	0.73 ± 0.10
Heart	6.8 ± 0.6	7.4 ± 0.8	6.1 ± 1.5	5.0 ± 1.2	6.6 ± 0.6
Eyes	0.23 ± 0.02	0.18 ± 0.04	0.17 ± 0.02	0.26 ± 0.04	0.34 ± 0.09
Brain	2.5 ± 0.2	1.9 ± 0.2	2.3 ± 0.1	2.2 ± 0.1	2.2 ± 0.1
Hindleg	1.3 ± 0.1	0.94 ± 0.6 P<0.05	0.60 ± 0.6 P<0.001	0.55 ± 0.1 P<0.001	0.72 ± 0.09 P<0.01
Hindpaw	0.22 ± 0.3	0.21 ± 0.02	0.09 ± 0.02 P<0.001	0.09 ± 0.03 P<0.001	0.16 ± 0.03
Tail	0.19 ± 0.02	0.33 ± 0.08 P<0.05	0.14 ± 0.03	0.07 ± 0.01 P<0.001	0.23 ± 0.03

TABLE 3

The effect of diabetes on the blood flow through various tissues (ml./100 gm./min. mean ± S.E.M.). Numbers per groups are indicated at the head of each column. N.D. = not done

Organ	Controls (15)	Streptozotocin-diabetic			Alloxan-diabetic
		3-day (8)	14-day (10)	60-day (5)	7-day (7)
Stomach	62 ± 7	59 ± 4	82 ± 16	111 ± 31 P<0.05	76 ± 5
S. Intestine	97 ± 12	153 ± 14 P<0.01	142 ± 17 P<0.01	151 ± 19 P<0.01	164 ± 16 P<0.01
L. Intestine	104 ± 14	137 ± 12	136 ± 17	143 ± 14	142 ± 8
Kidneys	613 ± 37	697 ± 43	613 ± 87	486 ± 54	487 ± 53
Liver	19 ± 2	25 ± 4	34 ± 8	15 ± 3	27 ± 5
Spleen	183 ± 22	153 ± 27	95 ± 41 P<0.05	69 ± 14 P<0.001	140 ± 16
Abdominal skin	9.9 ± 1.3	12 ± 2	6.2 ± 0.7	8.9 ± 1.6	8.9 ± 0.9
Hindleg skin	10 ± 1	13 ± 1	5.6 ± 0.7 P<0.01	8.2 ± 1.0	12 ± 2
Abdominal fat	10 ± 3	58 ± 14 P<0.001	N.D.	N.D.	61 ± 25 P<0.01
Abdominal muscle	13 ± 2	13 ± 2	18 ± 3	12 ± 2	13 ± 1
Adrenals	1,386 ± 168	1,057 ± 104	N.D.	914 ± 156 P<0.05	1,056 ± 228
Pancreas	223 ± 34	144 ± 15 P<0.05	N.D.	219 6c 52	166 ± 14

The hindleg skin of the 14-day streptozotocin-diabetic rats received a reduced blood flow (table 3).

The pancreatic blood flow was less than that of controls in the shortest-term diabetic group. That of the other diabetic groups in which it was measured was unchanged (table 3).

Blood flow through abdominal fat was markedly increased in the two shorter-term diabetic groups (table 3). In the longer-term diabetic groups too little fat was available for reliable measurement of blood flow.

None of the above changes were found to correlate significantly with either blood glucose or free fatty acid levels. However, the per cent CO received by the eyes of the 14-day streptozotocin-diabetic rats was found to be positively correlated with blood free fatty acid levels (figure 2), although no mean change of the former control values was observed (table 2).

Most of the changes observed appeared to be progressive, being greatest in the longest-term diabetic group. The decrease in pancreatic blood flow (table 3) and the increase in per cent CO received by the kidney and tail (table 2), however, appeared to be temporary, being absent or, in the case of the last mentioned, reversed in the longer term diabetic groups.

In most cases results from the seven-day alloxan-diabetic group fell between those from the three- and 14-day streptozotocin-diabetic groups, indicating a similar progression of events in both forms of dia-

TABLE 4

The effect of diabetes on organ weights as expressed as percentage body weight (mean ± S.E.M.). Numbers per group are indicated at the head of each column

Organ	Controls (15)	Streptozotocin-diabetic			Alloxan-diabetic
		3-day (8)	14-day (10)	60-day (5)	7-day (7)
Stomach	0.49 ± 0.02	0.55 ± 0.04	0.62 ± 0.06 P<0.01	0.8 ± 0.08 P<0.001	0.56 ± 0.02 P<0.05
S. Intestine	3.2 ± 0.1	3.7 ± 0.4	4.6 ± 0.4 P<0.001	6.0 ± 0.7 P<0.001	4.5 ± 0.3 P<0.001
L. Intestine	1.0 ± 0.05	1.1 ± 0.04	1.8 ± 0.2 P<0.001	2.4 ± 0.3 P<0.001	1.3 ± 0.1 P<0.05
Kidneys	0.69 ± 0.02	0.81 ± 0.14 P<0.01	1.1 ± 0.1 P<0.001	0.95 ± 0.08 P<0.001	0.92 ± 0.02 P<0.001
Liver	4.0 ± 0.1	3.9 ± 0.2	4.3 ± 0.2	5.1 ± 0.3 P<0.01	3.6 ±0.1 P<0.001
Spleen	0.28 ± 0.01	0.23 ± 0.02 P<0.05	0.20 ± 0.03 P<0.01	0.20 ± 0.02 P<0.001	0.22 ± 0.01 P<0.001
Adrenals	0.012 ± 0.001	0.015 ± 0.001	N.D.	0.020 ± 003	0.016 ± 0.001

betes. The per cent CO received by the kidneys was a notable exception, being reduced in both the aforementioned streptozotocin-diabetic groups but unchanged in the alloxan-diabetic group.

Effects of Genetic Obesity

The blood glucose levels of the obese rats were similar to those of their nonobese littermates. Blood free fatty acid levels were, however, elevated (table 1).

In these obese rats CO per unit weight was reduced compared with that of their nonobese littermates (figure 1).

Fat tissue of the obese rats received a reduced blood flow (table 5). Blood flows in the small intestine, liver, spleen, abdominal skin, and adrenals were also reduced. In no tissue examined was blood flow found to be increased.

DISCUSSION

The cardiovascular changes observed in pithed diabetic rats in an earlier study[13] were not reflected by any changes in CO in the anesthetized rats used in the present study. This does not, however, demonstrate normal cardiac performance. CO is not a good index of cardiac performance since even in the presence of major malfunctions it may be restored to normal by homeostatic reflexes.[20]

The increased per cent CO received by the stomach and intestines of the diabetic rats may, in part, be the result of the hyperglucagonemia and hyperglycemia. Glucagon has been shown to increase blood flow in these areas,[21] and raised perfusate glucose levels have been shown to reduce sympathetically mediated vasoconstriction in the perfused rat mesentery.[22] Hyperplasia of the tissues of the gastrointestinal tract, however, appears to be the major cause of the increased blood flow since blood flow per unit weight was, by comparison, only moderately increased. The increased weights of the small intestines of diabetic rats are in agreement with previous findings.[23,24] A homeostatic mechanism aimed at increasing food absorption in compensation for the loss of glucose in the urine may be responsible for this hyperplasia. Both alloxan- and streptozotocin-diabetic rats have been shown to consume significantly more food and water than controls, these changes paralleling increased loss of glucose and water in the urine.[25] Renal plasma flow is not substantially changed in early juvenile diabetes but glomerular filtration is elevated.[6] The latter may be of pathologic importance since blood flow restriction, which presumably reduces filtration, may afford protection from diabetic nephropathy.[10] The increased total renal blood flow of the two shorter-term

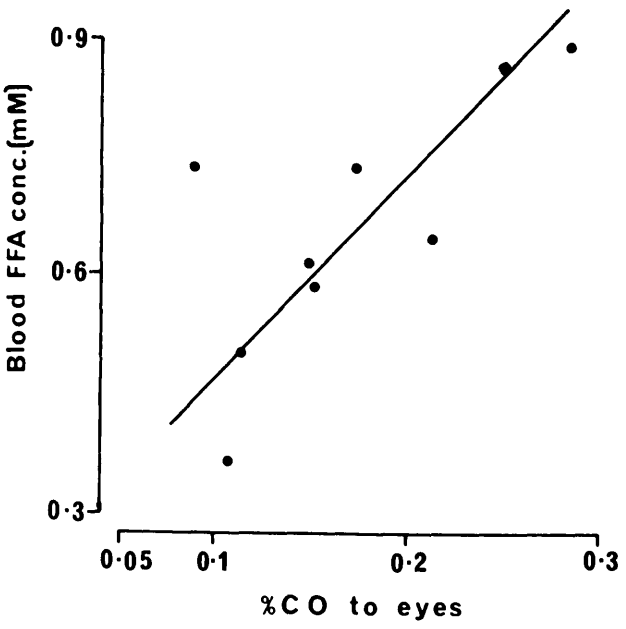

FIG. 2. Correlation between blood free fatty acids (FFA) and the percentage of cardiac output (% CO) received by the eyes of 14-day streptozotocin (60 mg./kg.)-diabetic rats. Regression coefficient = 0.395 per cent CO/mM FFA, and P<0.025.

streptozotocin diabetic groups of the present study may, therefore, produce kidney changes analogous to those of human diabetic nephropathy if, as in the human diabetic, a greater proportion of passing plasma is filtered. Kidney changes similar to those occurring in human diabetic nephropathy have been reported to occur in the streptozotocin-diabetic rat,[26] and their progression may be accelerated by procedures that further elevate blood flow in kidney

TABLE 5

Blood flow (ml./100 gm./min., ± S.E.M.) through various tissues of Zucker obese rats and their lean littermates. Numbers per group are indicated at the head of each column.

Organ	Lean littermates (7)	Zucker obese rats (8)
Stomach	63 ± 6	71 ± 7
S. Intestine	159 ± 14	89 ± 6
		P<0.001
L. Intestine	144 ± 14	122 ± 12
Kidneys	813 ± 81	557 ± 63
Liver	27 ± 3	13 ± 2
		P<0.005
Spleen	146 ± 26	90 ± 9
		P<0.05
Abdominal skin	6.1 ± 0.6	3.4 ± 0.5
		P<0.005
Abdominal fat	10.0 ± 1.5	4.8 ± 0.9
		P<0.01
Abdominal muscle	18 ± 4	13 ± 2
Adrenals	1,922 ± 133	1,113 ± 136
		P<0.001
Pancreas	154 ± 12	174 ± 18

tissue—e.g., unilateral nephrectomy. In the long-term diabetic with declining kidney function, renal blood flow and glomerular filtration rate are progressively reduced.[9] The reduction towards normal of the kidney flows of the longest-term streptozotocin diabetic rats of the present study may, therefore, indicate similar changes. Further studies aimed at correlating changes in blood flow with the progression of renal changes are in progress.

The decrease in per cent CO received by the spleen appeared to progress with the duration of diabetes and was so severe that, although a marked reduction in splenic weight occurred, blood flow per unit weight was also markedly reduced. The fact that the spleen normally contracts under the influence of anoxia may suggest that the diabetic rat spleen was anoxic. Anoxia resulting from reduced splenic blood supply, perhaps coupled with microcirculatory deterioration and increased blood oxygen affinity analogous to changes in the human diabetic,[1] may result in a more or less continuous splenic contraction. The progressive decrease in both splenic weight and blood flow may result in compromised functions of the spleen other than red cell storage.

Changes in retinal blood flow have been detected in the human diabetic, with increases in early diabetes being followed by a decline to normal if severe retinopathy develops.[5] No such changes of whole-eye blood flow were observed in the present study. This may be due to opposing blood flow changes in parts of the eye other than the retina or simply the masking of any changes by the large variations obtained within groups. The latter may be due to the large experimental errors inherent in the measurement of *small* blood flows by the microsphere technique.[12] Another factor contributing to eye blood flow variation was its correlation with blood free fatty acid levels. This correlation may be due to a reduction in blood pH by free fatty acids and, perhaps, their metabolites, ketone bodies. Reduced blood pH has previously been suggested to increase retinal blood flow.[28] Pancreatic B-cell necrosis followed by gradual absorption of the necrotic tissue may contribute to the temporary reduction of pancreatic blood flow.

Increased blood flow in fat has been found in the human diabetic when deprived of insulin and is reduced to normal by insulin administration.[2] Since fat blood flow is also reduced in normal subjects during starvation, metabolic causes related to increased fat mobilization are indicated. This hypothesis is supported by the present results in both diabetic and genetically obese rats. Marked increases in fat blood flow were observed in short-term diabetic animals and increased fat mobilization was illustrated by progressive reductions in fat tissue with the duration of diabetes. The latter resulted in too little fat for reliable measurement available in the longer-term diabetic animals. The fat of the genetically obese rats, in contrast, undergoes much lower than normal rates of catabolism.[15] This was reflected by reduced fat blood flows in these animals.

The increased fat blood flow in the diabetic animals coupled with rapid fat mobilization may explain the biphasic blood flow changes observed in their tails. Decreased blood flow in nonfat tissue may have become evident only after fat was severely depleted. A similar effect may also have occurred in the hindleg and paw and contributed to the greater blood flow depression observed in these organs in the longer-term diabetic animals. In most cases the degree of change in the seven-day alloxan-treated group falls between those of the three- and 14-day streptozotocin-treated rats, indicating similarly progressing changes. The changes observed, therefore, appear to be the result of the diabetic state rather than a direct effect of the diabetogens. The kidneys appear to be exceptions, with no increase in the per cent CO received by those of the seven-day allozan-treated despite increases in those of the three- and 14-day streptozotocin-treated rats. This may be due to the direct nephrotoxic effects of alloxan.[29]

The reductions in the per cent CO received by the tails and hindlimbs of the longer-term diabetic rats confirm the redistribution of blood supply suggested by a previous study.[13]

Increased resistance to blood flow and increased sensitivity to the constrictor effects of epinephrine have previously been demonstrated in perfused hindquarters of diabetic rats.[30] Glucocorticoids, whose levels have been reported to be raised in the diabetic rat,[31] may increase norepinephrine responsiveness by inhibiting catechol-0-methyltransferase.[32] Hyperglucagonemia may contribute to raised blood levels of glucocorticoids[33] and may therefore decrease hindquarter blood flow both by increasing mesenteric blood flow and promoting hindlimb vasconstriction. The latter effect might be expected to be greatest when sympathetic tone is increased. This may explain the report that diabetic rats are much more likely to develop frostbite in their tails when subjected to low temperatures.[14] Increased sympathetic outflow resulting from cold exposure would be expected to have a greater than normal constrictor effect on the vessels of the hindquarters, including those of the tail. The low-

ered constrictor sensitivity of other areas, such as the mesentery, due to hyperglucagonemia[21,34] and perhaps hyperglycemia,[22] would tend to reinforce sympathetically mediated constriction of the more responsive hindquarter vessels by means of homeostatic reflexes.

In conclusion, the technique of the regional blood flow measurement with radioisotopically labeled microspheres was shown to demonstrate changes in the diabetic rat that may be analogous to those occurring in the human diabetic with vasculopathy. Renal and hindlimb blood flow changes were of particular interest in this respect. Since a metabolic condition that is very similar to human diabetes was responsible for these changes, suitable experimental models of some aspects of human diabetic vasculopathy may have been demonstrated.

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