Glucose Metabolism in Leucocytes from Patients with Diabetes Mellitus, with and without Hypercholesteremia

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SUMMARY

The metabolism of glucose by the Embden-Meyerhof, pentose cycle and citric acid cycle pathways was determined in human polymorphonuclear leucocytes from control subjects and from patients with diabetes mellitus, with and without hypercholesteremia.

In controls over 85 per cent of glucose uptake was metabolized to lactic acid by the Embden-Meyerhof pathway; about 5 per cent of glucose uptake was recovered as CO_2, mostly from oxidation in the pentose cycle. Minimal citric acid cycle activity, a finding which correlated well with the paucity of mitochondria in the leucocyte, was shown by the slight and similar degree of oxidation of pyruvate and acetate.

Glucose metabolism in leucocytes from diabetics differed in two respects. The per cent of glucose uptake oxidized in the pentose cycle was increased in leucocytes from all diabetics. In the insulin-treated diabetics glucose uptake and lactic acid formation were similar to controls. In contrast, glucose uptake and lactic acid formation were reduced in leucocytes from adult, latent, hypercholesteremic diabetics. Pyruvate and acetate oxidation was similar in diabetics and controls. Thus, in terms of leucocyte metabolism, hypercholesteremia in the human diabetic was correlated with reduced glycolysis and not with reduced pentose cycle activity. DIABETES *14*:584-90, September 1965.

The ease with which human leucocytes can be obtained and separated from other blood components enhances their use as an intact human cell system for metabolic studies. Leucocytes differ from other body cells in that they have the capacity to migrate, contain few mitochondria, and metabolize glucose primarily to lactic acid. Glucose oxidation by the pentose pathway has been shown by Beck,[1] Buchanan,[2] and Marks, Gelhorn, and Kidson[3] demonstrated that leucocytes have the capacity to incorporate acetate-1-C-14 into lipids. Marks and associates[3] emphasized that lipid synthesis by leucocytes was enhanced by the presence of plasma, and that leucocyte lipids appeared rapidly in plasma. These observations suggested that leucocytes, and perhaps other blood cells, may contribute significantly to circulating lipids.

Human diabetes mellitus is the result of an inherited defect expressed in abnormalities of carbohydrate, lipid, and protein metabolism. Metabolic alterations have been demonstrated in liver, adipose tissue, skeletal and heart muscle and other tissues. Siperstein[4] has suggested an intimate relationship between glucose and cholesterol metabolism in diabetes. The human leucocyte might be expected to reflect the metabolic alterations present in diabetes mellitus. Studies of glucose uptake and lactic acid formation in leucocytes from patients with diabetes mellitus have produced variable results.[5-8] Awai, Hammarstrand, and Hennes[9] showed that the incorporation of acetate-1-C-14 into lauric, myristic, and palmitic acids was reduced in whole blood from patients in diabetic acidosis; they suggested that blood cells might be useful for studying certain aspects of lipid metabolism in human diabetics. Glucose oxidation by the pentose and citric acid cycle pathways has not been studied in leucocytes from diabetics. The purpose of this study was to determine the relative metabolism of glucose by the Embden-Meyerhof, pentose, and citric acid cycle pathways in leucocytes from controls and from patients with diabetes mellitus, with or without hypercholesteremia.

METHODS AND MATERIALS

Radiopurity of C-14-labeled substrates* (glucose-U-C-14, glucose-1-C-14, glucose-6-C-14, pyruvate-2-C-14, and acetate-1-C-14) was documented by paper

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*New England Nuclear Corporation, Boston 18, Mass.

chromatography. Dextran* (viscosity number 0.42, Mw 267,000, Lot No. TO 5887, Batch FDR 887) was used for erythrocyte sedimentation. Sterile nonpyrogenic water† and isotonic saline were used in all experiments. All glassware was siliconized, rinsed, and depyrogenated.[7] Apparatus and chemicals which could not be depyrogenated were sterilized by autoclaving. Phosphate buffer[7] was prepared by diluting 10 ml. of Stock A (75.0 g NaCl, 7.5 g KCl, 1.0 g Na_2HPO_4, 1.2 g KH_2PO_4, and 0.5 g K_2HPO_4 per liter) and 7 ml. of Stock B (10.0 g Na_2HPO_4 per liter) to 100 ml. with water. The final *p*H was 7.40 ± 0.05.

Pertinent clinical and laboratory features of leucocyte donors are shown in table 1. Patients who required insulin decreased by one half their usual dosage twenty-four hours before blood was obtained. On the day of venepuncture, no patient received insulin, and no acetonuria was present. In all subjects, blood was drawn after an overnight fast. From 400 to 600 ml. of blood were used for each experiment.

Blood was drawn directly into a Fenwal blood-pack which contained a final concentration of 0.04 mg. heparin sodium per milliliter of blood. Fifty milliliters of whole blood were added immediately to 12.5 ml. of 0.85 per cent NaCl containing 5 per cent dextran in a 100 ml. graduated cylinder. The cylinder was covered with Parafilm, inverted slowly several times, and refrigerated at 5° C. for forty-five minutes. After the erythrocytes had sedimented, the supernate was transferred to a 250 ml. round bottom centrifuge tube and centrifuged at 50 g for fifteen minutes at 4° C. The supernate was discarded, and the cell button was resuspended by gentle agitation in phosphate buffer. Twice the volume of distilled water was added to hemolyze the erythrocytes. Glucose uptake, lactic acid production, and C-14-O_2 production from C-14 glucose, labeled in the 1 or 6 positions, were identical in leucocyte preparations separated with, and without, the use of distilled water; the leucocyte/erythrocyte ratio was 5:1 with, and 1:5 without, separation using distilled water. That glucose metabolism was not detectably different with the increased percentage of erythrocytes was in keeping with the much greater capacity of leucocytes to metabolize glucose. After recentrifugation at 50 g for fifteen minutes at 4° C., the button of cells was resuspended in phosphate buffer. Quadruplicate cell counts were done using two pipettes and Spencer Brite-line Hemocytometers. The volume was adjusted so that each milliliter contained 45×10^6 leucocytes. The final leucocyte suspension consisted of over 90 per cent polymorphonuclear cells with a ratio of approximately one erythrocyte for each five leucocytes.

Two milliliters of the leucocyte suspension were added to 25 ml. Erlenmeyer flasks. One milliliter of buffer, containing substrate, was added making a total volume of 3 ml. and a total of 9×10^7 leucocytes per flask. Each flask was capped with a rubber vaccine port from which was suspended a stainless steel holder encircling a glass center well.[10] Each flask was gassed immediately for fifteen seconds with 100 per cent oxy-

*Pharmacia, Uppsala, Sweden.

†Baxter Laboratories, Inc., Morton Grove, Ill.

TABLE 1

Human leucocytes: Donors*

	Controls	Diabetes mellitus Normocholesteremia	Diabetes mellitus Hypercholesteremia
Number	16	6	9
Number receiving insulin	0	6	0
Age in years	26 (21-40)	26 (21-30)	49 (30-65)
Weight in pounds	130 (100-185)	125 (100-175)	150 (105-230)
Serum cholesterol, mg. per cent	210 (158-260)	184 (140-255)	423 (315-596)
Blood glucose, mg. per 100 ml.			
fasting	88 (80-95)	218 (52-300)	95 (85-106)
2 hr. after 100 gm. glucose	85-105	—	142-244
Insulin (units per day)	0	35-60	0
Duration of known diabetes (years)	0	4-10	0
Family history of diabetes	0	6	9

*The concentration of blood glucose was determined on all donors who did not require insulin two hours after 100 gm. of dextrose orally. Fasting blood glucose and serum cholesterol concentrations were determined on the day blood was obtained for leucocyte studies. Ranges are shown in parentheses.

gen at a flow rate of 2 liters per minute. A Dubnoff metabolic shaker with 15 oscillations per minute and a constant temperature of 37° C. was used for incubation. At the end of incubation, 0.3 ml. of Hyamine 10-X (p-[diisobutylcresoxyethoxyethyl]-dimethylbenzylammonium hydroxide) was added to the center well with a needle and syringe. Three milliliters of 1 M perchloric acid were added to the flasks with a needle and syringe to stop the reaction and liberate the carbon dioxide. The incubation was continued for an hour to insure that over 98 per cent of the carbon dioxide was trapped. Each center well was placed directly into a liquid scintillation vial which contained 10 ml. of a scintillator solution: 6.0 g POP (2,5-diphenyloxazole) and 0.4 g dimethyl POPOP (1,4 bis-2-[4-methyl-5-phenyloxazolyl]-benzene) per liter of toluene.

The contents of each flask were neutralized with potassium hydroxide, and the concentration of lactic acid and glucose was determined. Lactic acid was measured by the enzymatic method of Horn and Bruns.[11] Glucose uptake was calculated from the difference between the initial and final concentrations of glucose measured by the Somogyi-Nelson method.[12] Eight to sixteen flasks were used for each experiment. All experiments were paired with controls, or were done on alternate days.

Radioactivity measurements, using a liquid scintillation spectrometer, were corrected for quenching by the channels ratio method,[13] and for spontaneous decarboxylation. Results were expressed as micromoles of substrate per 9×10^7 leucocytes per four hours. Expressed as per cent, specific yield equaled micromoles of glucose recovered as C-14-O_2 divided by glucose uptake. Pentose pathway activity was calculated from the formula of Katz and Wood[14]:

$$\frac{Gl_{CO_2} - G6_{CO_2}}{1 - G6_{CO_2}} = \frac{3\ PC}{1 + 2\ PC}.$$

RESULTS

Glucose uptake, lactic acid and carbon dioxide production, and oxygen uptake by leucocytes from controls were essentially linear for four hours (figure 1). During the first ninety minutes of incubation, the rate of oxygen uptake was slightly less, and that of glucose uptake and lactic acid formation greater, than in subsequent periods.

Glucose uptake, lactic acid and carbon dioxide formation were maximal with a glucose concentration of 2.5 mM (table 2); at this concentration all of the glucose present was extracted. With glucose concentrations above 5 mM the uptake of glucose, and lactic acid formation, were less. All glucose extracted was accounted for by the lactic acid and C-14-O_2 (figure 1 and table 2). In other experiments glucose metabolism by the pentose pathway was determined. Leuco-

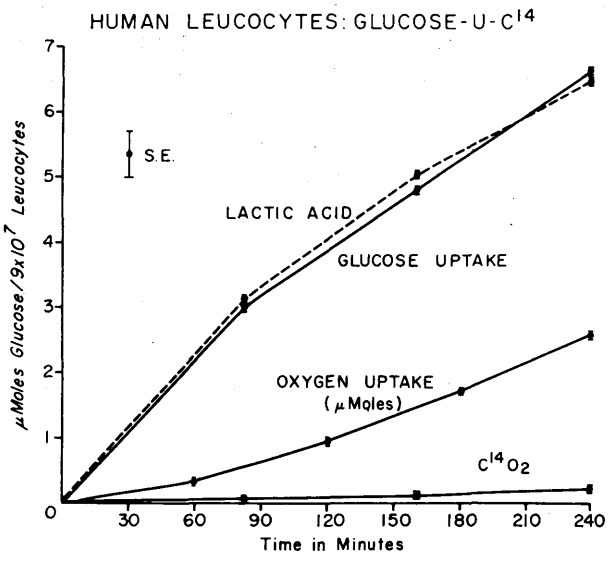

FIG. 1. Leucocytes were incubated in buffer containing 5 mM glucose-U-C-14. Glucose uptake, lactic acid, and C-14-O_2 were expressed as μmoles of glucose. Oxygen uptake, determined in Warburg manometers, was expressed as μmoles of oxygen per 9 X 10^7 leucocytes. Mean ± S.E. is shown.

TABLE 2

Human leucocytes: Effect of glucose concentration on glucose metabolism*

Glucose concentration mM	Flasks	Glucose uptake μmoles	Lactic acid μmoles	C-14-O_2 μmoles	Recovery (per cent)
2.50	8	7.33 ±0.10	6.89 ±0.05	0.243 ±0.006	97
5.00	5	7.17 ±0.22	7.14 ±0.11	0.265 ±0.012	103
10.00	5	6.20 ±0.10	6.61 ±0.05	0.233 ±0.006	110
20.00	6	5.61 ±0.24	6.63 ±0.09	0.257 ±0.007	122

*Leucocytes were incubated for four hours in buffer containing glucose-U-C-14. Glucose uptake, lactic acid and C-14-O_2 were expressed as μmoles of glucose per 9 × 10^7 leucocytes per four hours. Mean ± S.E. is shown. Recovery of glucose was calculated from the sum of lactic acid and C-14-O_2 divided by glucose uptake.

cytes from control subjects were incubated with either 5 mM glucose-1- or 6-C-14. From the C-14-O_2 formed, glucose oxidation by the pentose pathway was calculated by the method of Katz and Wood.[14] A similar percentage of the glucose uptake was metabolized in the pentose pathway by leucocytes incubated for one (0.41 ± 0.05 per cent) or four hours (0.51 ± 0.05 per cent).

The physiologic concentration of glucose (5 mM) was used in all subsequent experiments because glucose uptake was optimal at this concentration. An incubation period of four hours was chosen because: (1) Glucose uptake was linear over this time interval. (2) Glucose concentration decreased by 50 to 60 per cent which made accurate measurements of uptake possible and yet minimized the effects of changes in concentration. (3) Pentose pathway activity was unchanged with time.

In leucocytes from sixteen controls (table 3), the glucose uptake of glucose-1-C-14 (9.27 ± 0.55) and glucose-6-C-14 (8.93 ± 0.53) was the same. Lactic acid and C-14-O_2 production were dependent on glucose uptake. About 85 per cent of the glucose uptake was accounted for as lactic acid. C-14-O_2 accounted for 4.13 ± 0.11 and 2.66 ± 0.19 per cent of the glucose uptake from glucose-1-C-14 and glucose-6-C-14, respectively. The calculated per cent of glucose uptake metabolized by the pentose pathway was 0.51 ± 0.06 per cent.

Glucose uptake, lactic acid production, and C-14-O_2 were equal in leucocytes from controls and normocholesteremic diabetics (table 3). In contrast, glucose uptake, lactic acid production, and C-14-O_2 were less in leucocytes from hypercholesteremic diabetics. Compared with controls, the ratio of specific yields of C-14-O_2 and the calculated per cent of glucose uptake metabolized by the pentose pathway were greater in leucocytes from all patients with diabetes mellitus.

The studies with pyruvate and acetate were done to determine whether the small amount of glucose oxidized to carbon dioxide by nonpentose pathways was due to impaired decarboxylation of pyruvate or limited citric acid cycle activity. Essentially no oxidation of pyruvate was demonstrable at the physiologic concentration of 0.1 mM (table 4); definite oxidation was observed with 5 and 10 mM pyruvate. Acetate oxidation and incorporation of acetate into leucocyte lipids were minimal with 1, 5 and 10 mM acetate (table 5). No difference in pyruvate oxidation or conversion to lactate, or in acetate oxidation, was demonstrable in leucocytes from normocholesteremic insulin-treated diabetics, as compared with controls (table 6).

DISCUSSION

Glucose metabolism in leucocytes from controls

The linear increase in glucose uptake, lactic acid production, and carbon dioxide production indicated that the cells were actively metabolizing glucose for the four-hour incubation period. Further evidence of their functional integrity was shown by the oxygen uptake, and by the pseudopodia formation, granulation, and ameboid movements, which were present before and after incubation.

The observed decrease in glucose uptake at the higher glucose concentration was greater than the decrease in lactic acid production. It was likely that the decrease in glucose uptake with higher glucose concentrations was, at least in part, due to the inherent problem of measuring a small difference between two large numbers encountered with the higher concentrations. Support for this suggestion was the recovery of over 100 per cent with 10 and 20 mM glucose; with 2.5 and 5 mM glucose all of the uptake was accounted for as lactic acid and C-14-O_2 (table 2).

When glucose uptake in this study was expressed as μmoles of glucose per ten million leucocytes per hour, the mean rate of glucose uptake (0.25 ± 0.02) in controls was similar to that (0.20 ± 0.02) reported by Martin and associates.[5] The mean rate of lactic acid production (0.21 ± 0.02), however, was slightly less than that (0.28 ± 0.03) found by Martin and associates.[5] This difference in lactic acid production was probably related to the different methods used to measure lactic acid; Martin and associates[15] stated that lactic acid, when measured enzymatically as in the present study, was less than when determined colorimetrically.

In confirmation of the results of others[5-8,16] the major fate of glucose in human leucocytes was lactic acid. If the only source of lactic acid was exogenous glucose, 85 per cent of the glucose uptake by leucocytes from controls could be accounted for as lactic acid. This agreed closely with the findings of Beck.[16] Using C-14 glucose, Beck accounted for approximately 87 per cent of the glucose uptake as labeled lactic acid.

The amount of glucose oxidized to carbon dioxide was small, but reproducible. If nontriosephosphate pathways were negligible, the C-14-O_2 recovered from glucose-1-C-14 represented the relative amount of glucose traversing the pentose, Embden-Meyerhof, and citric acid cycle pathways. Similarly, the C-14-O_2 re-

TABLE 3

Human leucocytes: Glucose metabolism in diabetes mellitus*

Donors		Glucose uptake		Lactic acid	
Type	Number	C_1 μmoles	C_6 μmoles	C_1 μmoles	C_6 μmoles
Controls	16	9.27 ±0.55	8.93 ±0.53	7.88 ±0.43	7.33 ±0.43
Diabetes mellitus, normocholesteremia	6	10.48 ±0.61 (>0.3)	10.33 ±0.79 (>0.3)	9.10 ±0.99 (>0.3)	8.88 ±0.89 (>0.3)
Diabetes mellitus, hypercholesteremia	9	6.40 ±0.45 (<0.001)	6.35 ±0.45 (<0.001)	5.94 ±0.38 (<0.01)	5.88 ±0.34 (<0.01)

*Leucocytes were incubated for four hours in buffer containing 5 mM glucose-1-C-14 (C_1) or glucose-6-C-14 (C_6). Glucose uptake, lactic acid and C-14-O_2 were expressed as μmoles of glucose per 9×10^7 leucocytes per four hours. Specific yield, expressed as per cent, equalled the C-14-O_2 formed divided by the glucose uptake. Recovery was computed from the sum of lactic acid plus C-14-O_2, divided by glucose uptake. Mean ± S.E. is shown. P values, shown in parentheses, were obtained by comparing experimental group with control group using Student's *t* test.

†Glucose-1-C-14 specific yield divided by glucose-6-C-14 specific yield.

‡Calculated by the formula of Katz and Wood:[14]

$$\frac{G_1CO_2 - G_6CO_2}{1 - G_6CO_2} = \frac{3\ PC}{1 + 2\ PC}$$

covered from glucose-6-C-14 represented the amount of glucose metabolized by the Embden-Meyerhof and citric acid cycle pathways. Using the formula of Katz and Wood,[14] 0.51 ± 0.06 per cent of the glucose uptake was metabolized by the pentose pathway in leucocytes from controls. This agreed closely with the estimate of glucose oxidation by the pentose pathway reported by Beck.[1]

TABLE 4

Human leucocytes: Pyruvate-2-C-14 oxidation*

Concentration of pyruvate-2-C-14 mM	Donor	C-14-O_2 μmoles	
0.1	1	0.06 ± 0.01	(5)
	2	0.06	(2)
5.0	1	2.75 ± 0.06	(6)
	2	1.29 ± 0.04	(7)
	3	1.59 ± 0.04	(6)
	4	1.58 ± 0.05	(3)
	5	1.24 ± 0.10	(3)
10	1	3.83 ± 0.13	(6)
	2	3.93	(2)

*Leucocytes from controls were incubated for four hours. Studies on leucocytes from donors 1 and 2 were done on the same day, and from donors 3, 4 and 5 on a different day. Results, expressed as μmoles of pyruvate per 9×10^7 leucocytes per four-hours incubation, show the mean ± S.E. Number of flasks is shown in parentheses.

Glucose metabolism in leucocytes from patients with diabetes mellitus

Glucose metabolism in leucocytes from diabetics differed in two respects. In all diabetics the per cent of glucose uptake oxidized in the pentose pathway was increased. Only in the adult-onset, or latent diabetics

TABLE 5

Human leucocytes: Acetate-1-C-14 metabolism*

Concentration of acetate-1-C-14 μM	Donor	C-14-O_2 μmoles	Tissue lipids mμmoles
1	1	1.71±0.07 (3)	4.0±0.8 (3)
5	1	2.17±0.07 (4)	4.0±0.5 (4)
	2	2.22±0.08 (4)	—
	3	2.54±0.16 (3)	—
10	1	1.91±0.13 (4)	6.1±1.1 (4)

*Leucocytes from controls were incubated for four hours. Radioactivity in lipids was determined on a chloroform: methanol extract (Folch). Studies with donor 1 were done on the same day, and those with donors 2 and 3 on separate days. C-14-O_2 was expressed as μmoles of acetate and C-14 lipids as mμmoles of acetate per 9×10^7 leucocytes per four-hour incubation. Mean ± S.E. is shown. Number of flasks is shown in parentheses.

TABLE 3 (*continued*)

Human leucocytes: Glucose metabolism in diabetes mellitus*

C-14-O_2				$\frac{C_1 \text{ S.Y.}\dagger}{C_6 \text{ S.Y.}}$	Pentose pathway‡ (per cent)	Recovery (per cent)	
C_1		C_6				C_1	C_6
μmoles	S.Y. in per cent	μmoles	S.Y. in per cent				
0.383 ±0.023	4.13 ±0.11	0.237 ±0.023	2.66 ±0.19	1.55 ±0.07	0.51 ±0.06	89.1 ±2.6	89.2 ±3.1
0.425 ±0.033 (>0.9)	4.05 ±0.32 (>0.9)	0.210 ±0.018 (>0.3)	2.03 ±0.02 (>0.3)	2.00 ±0.13 (>0.01)	0.70 ±0.04 (>0.02)	90.8 ±5.5	87.9 ±5.2
0.213 ±0.021 (<0.001)	3.34 ±0.23 (<0.05)	0.085 ±0.014 (<0.001)	1.34 ±0.16 (<0.001)	2.49 ±0.20 (<0.001)	0.69 ±0.07 (<0.05)	96.1 ±2.9	93.9 ±2.8

*Leucocytes were incubated for four hours in buffer containing 5 mM glucose-1-C-14 (C_1) or glucose-6-C-14 (C_6). Glucose uptake, lactic acid and C-14-O_2 were expressed as μmoles of glucose per 9 × 10^7 leucocytes per four hours. Specific yield, expressed as per cent, equalled the C-14-O_2 formed divided by the glucose uptake. Recovery was computed from the sum of lactic acid plus C-14-O_2, divided by glucose uptake. Mean ± S.E. is shown. P values, shown in parentheses, were obtained by comparing experimental group with control group using Student's *t* test.

†Glucose-1-C-14 specific yield divided by glucose-6-C-14 specific yield.

‡Calculated by the formula of Katz and Wood:[14]

$$\frac{G_1CO_2 - G_6CO_2}{1 - G_6CO_2} = \frac{3 \text{ PC}}{1 + 2 \text{ PC}}$$

TABLE 6

Human leucocytes: Pyruvate (5 mM) and acetate (5 mM) metabolism in patients with diabetes mellitus*

Donors	Pyruvate-2-C-14 C-14-O_2 μmoles	Pyruvate-2-C-14 Lactic acid μmoles	Acetate-1-C-14 C-14-O_2 μmoles
Controls			
1	1.29±0.04 (7)	—	—
2	1.59±0.04 (6)	10.67±0.13 (6)	—
3	1.58±0.02 (3)	7.64±0.18 (3)	2.22±0.08 (4)
4	1.24±0.10 (3)	4.77±0.12 (3)	2.54±0.16 (3)
Mean± S.E.	1.43±0.08	8.01±1.40	2.38
Diabetes mellitus, normocholesteremia			
1	1.08±0.04 (6)	—	—
2	1.04±0.04 (6)	8.24±0.13 (6)	—
3	1.72±0.02 (6)	7.90±0.13 (6)	3.55±0.04 (7)
4	1.34±0.05 (6)	5.74±0.33 (6)	2.57±0.03 (5)
Mean± S.E.	1.30±0.16	6.97±0.90	3.06

*Leucocytes from control and diabetic donors (insulin-treated) of the same number were studied on the same day. Results were expressed as μmoles of pyruvate or acetate per 9 × 10^7 leucocytes per four-hour incubation. Mean ± S.E. on each donor, and for each group of donors, is shown. Number of flasks is shown in parentheses.

with hypercholesteremia was there a reduction in glucose uptake, and comparable decrease in lactic acid formation.

Previous studies of glucose uptake and lactic acid formation by leucocytes from diabetics have produced variable results. In contrast to the findings in the insulin-treated diabetics of this study, Dumm[6] found that glucose uptake was decreased in leucocytes (obtained twenty-four hours after insulin) from patients with diabetes mellitus who required more than 30 U. of insulin daily; in her study a decrease in lactic acid production was not demonstrated. Esmann,[8] on the other hand, was unable to show a difference in glucose uptake by leucocytes from untreated or insulin-treated diabetics. However, in his study, glucose uptake was increased by insulin, in vitro and in vivo. Also, in vitro insulin responsiveness of the leucocyte has been shown by Martin and associates[5] and Dumm.[6]

In leucocytes from the hypercholesteremic latent diabetics, who were older and weighed more than the insulin-dependent group, glucose uptake and lactic acid formation were reduced. The decrease in glucose uptake was probably the primary difference since lactic acid formation varied directly with glucose uptake. Whether the change in glucose uptake was a manifestation of the diabetic state or in some way related to

the disturbance of lipid metabolism was not directly investigated. A comparison of glucose metabolism in leucocytes from normocholesteremic and hypercholesteremic untreated diabetics would provide information on this point.

Pentose pathway activity was increased in leucocytes from all patients with diabetes mellitus. This increase was not related to glucose uptake, duration of incubation, lactic acid production, or peripheral blood concentration of cholesterol or glucose.

C-14-O_2 formation, expressed in carbon equivalents, was similar with 5 mM pyruvate-2-C-14 and 5 mM acetate-1-C-14, concentrations at which CO_2 formation was maximal. This finding suggested that the limited oxidation of both substrates was related to reduced citric acid cycle activity and not to a defect in pyruvate decarboxylation. The small amount of citric acid cycle activity was in agreement with the findings of Beck,[16] probably reflecting the paucity of mitochondria in human polymorphonuclear leucocytes. No difference was demonstrated in pyruvate or acetate oxidation between leucocytes obtained from normocholesteremic, insulin-treated diabetics and controls. These results suggested that the citric acid cycle in the leucocytes was not altered by diabetes mellitus. The minimal lipid synthesis from acetate was probably explained by the absence of plasma, a requirement for lipid synthesis by leucocytes.[3]

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