Glucose Utilization and Lactate Production by Leucocytes of Patients with Diabetes Mellitus.* (23294)

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White blood cells are a readily available tissue for the study of cellular reactions in human subjects. Their metabolism has been extensively studied in cells from normal subjects and from patients in a variety of clinical situations, particularly patients with leukemia (1,2,3,4). Martin *et al.*(5) reported that glucose utilization and lactate production are lower in leucocytes from patients with diabetes mellitus than in normal human subjects.

In the present studies utilization of glucose and production of lactate by leucocytes and the effect of insulin on these reactions have been studied in patients with diabetes mellitus and in nondiabetic human subjects.

Procedures. The 9 diabetic subjects attended Third Medical Divison Diabetic Clinics at Bellevue Hospital. They ranged from 11 to 58 years and all were fasting when blood specimens were taken. All the diabetic subjects required at least 30 units of insulin/day in the form of regular or NPH insulin. In most cases, an interval of at least 24 hours had elapsed from time of injection of insulin until blood sample was taken. The 13 nondiabetic subjects were medical students or laboratory personnel between 20 and 45 years of age with no known metabolic disease and no family history of diabetes. Experiments were done on both fasting and non-fasting subjects. In 3 cases, blood from non-fasting subjects was obtained during a glucose tolerance test. Venous blood specimens of 30 to 50 ml were obtained using silicone-treated syringes and sodium citrate or heparin as an anticoagulant. Six experiments were run in duplicate on heparin-treated and citrate-treated blood. Level of blood sugar was determined in each specimen and total number of white blood cells counted. To increase sedimentation rate of the erythrocytes, dextran (average molecular weight 194,000) was added to the blood to a concentration of 0.3 g%. Ascorbic acid was added to a concentration of at least 1.5 mg %(6). Glucose was added in varying amounts to the blood specimens from normal subjects to final concentrations of 115 to 260 mg %. The erythrocytes settled out in the cold, usually in about an hour, and the plasma containing the suspended leucocytes was drawn off. As found by others, the leucocyte suspension always contained erythrocytes(6). On the average, there were approximately equal numbers of red and white blood cells present in the plasma suspensions. This degree of contamination is not serious in these experiments since the glycolytic rate of the leucocytes/cell is about 100 times more rapid than that of the erythrocytes(3). *Measurement of glucose utilization and lactate production*: Aliquots of the leucocyte suspension were transferred to small silicone-treated flasks for measurement of glucose utilization and lactate production. The flasks were shaken under oxygen or air(1) in water bath at 37°C for 2 to 2½ hours. Concentration of glucose was measured by the Somogyi-Nelson method (7,8) and lactate by the method of Barker and Summerson(9). The number of white blood cells present was determined by counting at least 2 pipettes in quadruplicate for each leucocyte suspension. The distribution of the various types of leucocytes was determined by differential white cell counts. In experiments on the effect of *in vitro* insulin, insulin was added to the white cell suspensions in a small volume of sterile isotonic saline. Both glucagon-free and regular crystalline zinc insulin (Lilly) were used. In all experiments, comparable volumes of saline were added to other aliquots of the leucocyte suspensions as controls.

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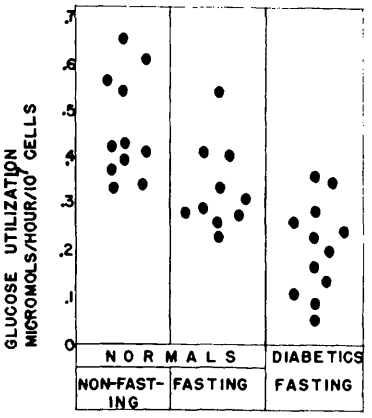

FIG. 1. Rate of glucose uptake by leucocytes of normal subjects and of patients with diabetes mellitus shown in μmols/hr/10 million cells at 37°C. Each point represents one experiment.

Results. The rates of glucose utilization and lactate production by leucocytes from normal and diabetic human subjects are summarized in Fig. 1. In the fasting normal subjects, the average rate of glucose utilization was 0.35 ± 0.03† μmole hour/10 million cells. In the non-fasting subjects, the rate was 0.45 ± 0.04 μmole/hour. In the diabetic patients who had taken no insulin for 24 hours, the mean rate of glucose utilization was 0.22 ± 0.04 μmole/hour. The difference in rate of glucose utilization between either group of normal subjects and the diabetic subjects is significant by the Fisher "t" test (diabetics compared with fasting normals $p<0.05$; with non-fasting normals $p<0.01$). The difference between fasting and non-fasting normal subjects is suggestive but does not meet the statistical criteria of significance. No significant difference in lactate production was observed between any of the groups tested. Lactate production averaged 0.79 ± 0.11 μmole/hour/10 million cells from fasting normal subjects, 1.01 ± 0.13 μmole/hour by cells from the non-fasting normals, and 0.81 ± 0.12 μmole/hour by cells from diabetic subjects.

Compared with heparin, sodium citrate was without significant effect on either glucose uptake or lactate production by the leucocytes. In 6 experiments on both normal and diabetic subjects, the average glucose uptake in heparin was 0.34 μmole/hour/10 million cells compared to 0.35 μmole/hour in sodium citrate. Values for lactate production were 0.92 μmole/hour/10 million cells in heparin and 0.87 μmole/hour in citrate.

Distribution of the various types of cells in the leucocyte suspensions was determined by differential white blood cell counts. The proportion of the various types of cells present was within normal limits and did not differ significantly in the 2 groups of subjects.

Effect of insulin in vitro: The absolute change in rate of glucose utilization and of lactate production when insulin (up to 0.5 unit ml) was added *in vitro* is shown for normal and for diabetic subjects in Fig. 2. An estimate of the probable error between duplicate experiments is also included in the Figure. This was computed as one standard deviation above and below the mean difference between duplicate experiments on 2 aliquots of the same leucocyte suspension. (The observed differences between duplicates were assigned positive or negative signs at random.) In about half of the experiments on normal subjects and in more than three-fourths of the experiments on diabetics, the addition of insulin to the leucocyte suspensions was associated with increases in glucose utilization greater than the probable difference between duplicate experiments. The data were also analyzed by the Fisher "t" test, comparing the mean change in rate of glucose utilization where insulin was added to the mean difference be-

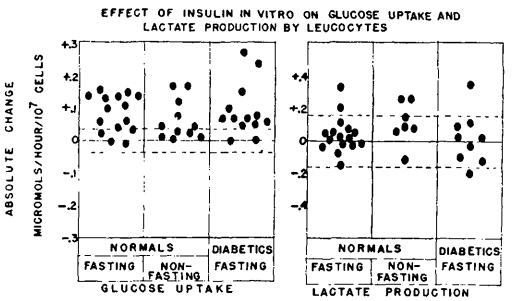

FIG. 2. Effect of adding insulin *in vitro* to leucocytes is shown as the absolute change in rate of glucose uptake and of lactate production in μmols /hr/10 million cells at 37°C. Dotted line indicates stand. dev. of the mean difference between duplicate determinations, when + or − signs were assigned at random to the differences.

† Mean ± Standard Error.

tween duplicate experiments. In both the normal and the diabetic subjects, the difference in glucose uptake when insulin was added was significant at the $p<0.01$ level. Insulin had no significant effect on lactate production as shown in Fig. 2.

Discussion. The data presented confirm the work of Martin *et al.*(5) and indicate that rate of glucose utilization by leucocytes is depressed in white blood cells of diabetic patients requiring insulin. In addition, the data suggest that leucocyte metabolism is responsive, in many instances, to *in vitro* insulin. The observation that leucocytes from nonfasting normal subjects tend to have higher rates of glucose utilization than do leucocytes from fasting normal subjects is consistent with considerable evidence that ingestion of carbohydrate normally results in the secretion of additional insulin. Martin *et al.*(5) reported that addition of insulin at concentrations up to 0.1 unit/ml increased glucose utilization and lactate production by leucocytes from diabetic subjects but did not influence these determinations on leucocytes from normal subjects. In our experiments, insulin concentrations up to 0.5 unit/ml were associated with significantly increased glucose utilization in 11 out of 13 determinations on leucocytes of diabetic subjects and in 14 out of 26 determinations on cells of normal subjects. The difference between the results of Martin *et al.* and those reported here is probably related to difference in the concentration of insulin used. Apparently leucocytes from diabetic subjects who require insulin are more likely to be sensitive to *in vitro* insulin than are leucocytes from normal subjects, particularly when the insulin is added at low concentrations.

The surviving tissue in which the effect of *in vitro* insulin has been most thoroughly studied is the rat diaphragm. While there are obvious differences in physical characteristics of rat hemidiaphragms and leucocyte suspensions, it is of interest to compare the rate of glucose utilization and the effect of insulin added *in vitro* to these 2 systems. If the data are expressed in the same units, assuming that 10 million human leucocytes weigh about 6 mg(3), the mean rate of glucose utilization by normal leucocytes incubated in plasma is 10 mg/g (wet weight) of tissue/hour at 37°C. Rate of glucose utilization by the normal rat diaphragm is about half as great, or 4 to 6 mg/hour(10,11,12). When insulin was added to the incubation medium, rate of glucose utilization was increased to about 13 mg/hour by the leucocytes (up to 0.5 unit of insulin/ml), and to 6 to 10 mg/hour by the rat diaphragm (up to 1.6 units/ml), in experiments reported by Gemmil and Hamman(10), Bornstein and Park(11) and Groen *et al.*(12).

There is no necessary contradiction in the observation that glucose utilization by leucocytes is enhanced by insulin and that lactate production is not. Leucocytes are known to contain considerable amounts of glycogen which can serve as a source of lactate(13). Shaw and Stadie(14) have reported experiments on the rat diaphragm which suggest that lactate production from glycogen is not responsive to insulin.

Summary. Glucose utilization and lactate production have been measured in suspensions of leucocytes in plasma from normal human subjects and from patients with diabetes mellitus. The rate of glucose utilization was significantly lower in leucocytes from severe diabetics than in leucocytes from normal human subjects. Lactate production did not differ significantly between normal and diabetic subjects. In more than three-fourths of the experiments on diabetics and about one-half the experiments on normal subjects, glucose utilization by leucocytes was increased by insulin *in vitro*. Lactate production was not increased when insulin was added *in vitro*.

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Vitamin A-Choline Interrelationship.* (23295)

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Abels, Gorham, Pack, and Rhoads(1) found that levels of vit. A in plasma of patients with malignant disease, particularly of the gastrointestinal tract, were below the normal range in 86% of cases examined. No response was obtained after vit. A administration, but feeding of yeast or pancreatic extracts, themselves free of carotenoids, raised the reduced plasma levels of vit. A. That this property in yeast or pancreas may be due to choline content has become apparent from later experiments. There was an average rise of 73% in plasma levels of vit. A in patients who were fed 1.5 g of choline chloride each day for 3 days. Popper and Chinn(2) showed that rats on a choline-poor diet containing liberal supplements of carotene developed fatty livers poor in vit. A. Therefore, it seemed worth while to determine choline levels of rat livers in normal, vit. A-supplemented, and vit. A-deficient animals.

Methods. In general, the procedure was to determine the amount of vit. A and choline in separate aliquots of rat livers from different dietary groups. Male, albino, Wistar-strain rats were used. The rats were divided into 3 dietary groups, normal (N) supplemented (S), and deficient (D). All animals were placed on a Red Range Dog Food† ration for at least 2 weeks. Animals of the supplemented group received an additional 5,000 International Units of vit. A‡ orally each day for one week before sacrifice. The deficient group was maintained on a vit. A test diet§ up to 7 weeks at which time vit. A analyses indicated that only trace amounts of vit. A remained in the liver. Each group was fasted for 24 hours before sacrifice by decapitation, accompanied by gravity exsanguination for approximately one minute. Usually 3 rats from the same dietary group were sacrificed at one time and their livers pooled. The livers were removed and placed in a freezer, at 10 to 15°C. The livers were then placed in a pre-cooled flask and weighed. The vit. A levels were run on freshly-chilled portions of liver, while total and phospholipid choline determinations were run on frozen livers. The livers, for vit. A analysis, were minced and one-half to one g portions were placed in a Potter-Elvehjem homogenizing tube containing 5 to 7 ml of ice-cold (0-5°C) distilled water. The homogenate was transferred to a 100 ml volumetric flask, and more liver was homogenized until 3 to 6 g of liver had been homogenized. The total homogenate was diluted to 100 ml with more cold distilled water. Triplicate aliquots of the homogenate were used immediately for determination of vit. A,

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† Red Range Dog Food sold by Southern States Feed Cooperative. Average Analysis (%), Protein 24.60, Fat 5.04, CHO 58.12; Vit. content/100 g, Vit. A activity-1100 I.U., Carotene 0.14 mg, Choline chloride 7.13 mg.

‡ Aqueous solution of vit. A prepared by Endo Products Inc. containing 25,000 I.U. of vit. A/ml of solution.

§ U. S. Pharmacopeia vit. A test diet distributed by Nutritional Biochemicals Corp. Average choline content 20 mg/100 g.