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Research Article

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Impaired Carbohydrate Metabolism of Polymorphonuclear Leukocytes in Glycogen Storage Disease Ib

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Abstract

This study measures hexose monophosphate (HMP) shunt activity, glycolytic rate, and glucose transport in PMN and lymphocytes of patients with glycogen storage disease (GSD) type Ib as compared with controls and with GSD Ia patients. HMP shunt activity and glycolysis were significantly lower in intact PMN cells of GSD Ib patients as compared with GSD Ia patients and with controls. These activities were above normal levels in disrupted GSD Ib PMN. HMP shunt activity and glycolytic rates in lymphocytes were similar in all three groups studied. The rate of 2-deoxyglucose transport into GSD Ib PMN was 30% of that into cells of normal controls. In GSD Ib lymphocytes or in GSD Ia PMN and lymphocytes transport was normal. The striking limitation of glucose transport across the cell membrane of the PMN of GSD Ib patients may account for the impairment of leukocyte function that is characteristic of GSD Ib, but not found in GSD Ia patients.

Introduction

Glycogen storage disease (GSD)¹ type I is a metabolic disease caused by a defect in the activity of glucose-6-phosphatase (D-glucose-6-phosphate phosphohydrolase, EC 3.1.3.9) (G6Pase), normally present in liver, kidney, and intestinal mucosa cells (1, 2).

Hepatic glucose-6-phosphatase is part of the multicomponent system of the endoplasmic reticulum (3). In 1975, Arion postulated that at least three components of the endoplasmic reticulum participate in the process of glucose-6-phosphate (G6P) hydrolysis: (a) a G6P specific translocase that shuttles G6P across the membrane; (b) a relatively nonspecific phosphohydrolase located on the luminal surface of the membrane; and (c) a translocase which mediates phosphate efflux out of the cisternae of the endoplasmic reticulum (4, 5).

Three groups of patients with GSD I have been identified:

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1. *Abbreviations used in this paper:* G6P, glucose-6-phosphate; GSD, glycogen storage disease; HMP, hexose monophosphate; KRP, Krebs-Ringer phosphate buffer.

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(a) GSD Ia patients, whose liver specimens lack the enzyme G6Pase (1); (b) GSD Ib patients, whose translocase for G6P in the microsomal membrane is defective (5, 6); and (c) GSD Ic patients, who lack the translocase for phosphate (7).

Patients with GSD Ib suffer from recurrent bacterial infections related to neutropenia and impairment of neutrophil functions (8, 9).

In most patients with GSD Ib, defects in random and direct cell migration (10, 11), bactericidal activity (12), phagocytosis (13), superoxide anion production (11, 14), and hexose monophosphate (HMP) activity (14) have been described. Since these functions all depend on an adequate supply of energy and NADP, we studied HMP shunt activity, glycolytic activity, ATP concentration, and glucose transport in PMN of three GSD Ib patients as compared with controls.

Methods

Patients

GSD was diagnosed on liver biopsies: GSD Ia by the absence of G6Pase activity, and GSD Ib by a high latency, i.e., low activity of G6Pase in fresh homogenates and normal activity in disrupted microsomes (15). The three GSD Ib patients suffered from numerous infections. The clinical and therapeutic data of the three GSD Ib patients and the one GSD Ia patient are summarized in Table I. There were no differences in the therapeutic management and the nutritional support of the patients. Studies of PMN were performed on preparations with initial cell counts of 600–1,500 cells/ μ l.

Cell preparation

PMN and lymphocytes were separated from heparinized venous blood by the Ficoll-Hypaque centrifugation methods (11). 20 ml fresh blood was mixed with 2 vol of 3% dextrane in 0.85% NaCl. After 1 h at 4°C the supernatant was layered on top of a Ficoll-Paque (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden) at a ratio of 6 ml of cell suspension to 3 ml of Ficoll. This was then centrifuged at 400 g for 30 min at 5°C. The interface layers were removed for lymphocyte preparation and the pellet for PMN preparation. Contaminating red cells were removed by the addition of 6 ml distilled H₂O for 20 s, and isotonicity was established by the addition of 2 ml 3.5% NaCl.

Cells were suspended in Krebs-Ringer phosphate (KRP) buffer and counted in a hemocytometer. Cell suspensions were then adjusted to 10×10^6 cells/ml.

Activity of the HMP Shunt

In intact cells. The activity of the HMP shunt was determined by a modification of the method described by Newburger et al. (16). Leukocytes were suspended in KRP (10^6 cells/ml) with 2 mM [1-¹⁴C]-glucose (0.5 μ Ci/ml) and 1 mM methylene-blue. Incubation was performed in sealed tubes with a central well containing filter paper impregnated with 200 μ l hyamine hydroxide. After 1 h of incubation at 37°C, 0.5 ml of 2 N H₂SO₄ was added to stop the reaction and to release ¹⁴CO₂ from the solution. The tubes were subsequently agitated for another 30 min. The filter paper was removed and placed in vials filled with a toluene-based scintillation fluid, and radioactivity was

Table I. Laboratory Data of the Patients Studied

	GSD Ib patients			GSD Ia
	L.S.	A.A.	N.T.	T.Y.
Age (yr)	13	4	3	3
Lactic acid (mM)	3.1	3.7	2.9	2.8
Uric acid (mg/dl)	4.7	7.0	5.7	7
Cholesterol (mg/dl)	98	128	173	230
Serum glutamic oxaloacetic transaminase (IU)	150	187	353	174
Serum glutamic pyruvic transaminase (IU)	108	95	230	168
Hb A ₁ C (%)	5.5	4.2	3.8	5.4
Total leukocytes (cells/mm ³)	3,100	5,700	5,500	13,000
PMN (% of total leukocytes)	24	27	15	28

determined in a scintillation spectrometer (Tricarb; Packard Instruments, Downers Grove, IL).

In disrupted cells. Cells were suspended in KRP containing 1% Triton-X100. HMP shunt activity was measured as described for intact cells, in the presence of 0.75 mM NADP and 0.75 mM ATP.

Glycolytic activity

In intact cells. Glycolytic activity was measured as described earlier (17): leukocytes ($1.5-3 \times 10^6$) were incubated at 37°C in a medium containing 100 mM Hepes buffer, 5 mM KCl, 80 mM NaCl, 1.2 mM MgSO₄, 2.5 mM CaCl₂, 0.2 mM NaH₂PO₄, and 2.5 mM glucose, pH 7.6, in a final volume of 0.5 ml. Lactate production was determined as described previously (18).

In disrupted cells. Cells ($1.5-3 \times 10^6$) were suspended in KRP containing 1% Triton X-100, 5 mM glucose, 1 mM ATP, and 0.7 mM NAD. Lactate production was measured as in intact cells.

Glucose transport

Uptake of 2-deoxyglucose was measured as previously described (19). 2×10^6 purified PMN or lymphocytes-monocytes were suspended in 1 ml KRP containing 5 μ Ci/ml [³H]2-deoxyglucose at concentrations indicated in the legends. Suspensions were incubated at 37°C, and uptake was stopped at various time intervals by adding 1 ml cold 0.9% NaCl. The samples were centrifuged at 3,000 g for 3 min and the pellets were washed twice with 2 ml cold 0.9% NaCl and centrifuged. The final pellet was dissolved in 0.5 ml water, and after repeated freeze-thawing procedures, samples were taken for determination of radioactivity and protein.

Phosphorylation of 2-deoxyglucose

PMNs (1×10^6) were incubated at 37°C in a shaking bath for different lengths of time in a final volume of 60 μ l containing 1 mmol/liter [³H]-2-deoxyglucose (0.2 μ Ci). The reaction was stopped by chilling in ice. The samples were centrifuged at 3,000 g for 2 min. The pellets were then washed three times in 0.9% wt/vol NaCl. The final pellets were suspended in 1 ml water and boiled for 5 min in a water bath. Subsequently, denatured protein was removed by centrifugation at 27,000 g for 10 min. The entire supernatant was applied to a small column of Dowex-1-Cl-resin (10 cm \times 1 cm) as previously described (19). The column was washed with 30 ml of H₂O to elute free 2-deoxyglucose, and fractions of 0.5 ml were collected. The column was then washed with 30 ml of 0.5 mol/liter HCl to remove deoxyglucose-6-phosphate, and 0.5-ml fractions were collected. Each fraction was added to 5 ml Instagel and the radioactivity was measured in a liquid scintillation spectrometer.

Osmotic fragility of PMN

0.4×10^6 cells were incubated at room temperature for 30 min in different NaCl concentrations (0–0.8% wt/vol). At the end of incubation, cells were centrifuged for 10 min at 3,000 g. Lactate dehydrogenase activity in the supernatant was measured as described (20).

Efflux of 2-deoxyglucose

10^6 cells were incubated in KRP with [³H]2-deoxyglucose (1.5 mM, 0.2 μ Ci) in a final volume of 60 μ l. Control cells were incubated for 20 min and patients' cells for 60 min. After subsequent washes in cold KRP, the cells were incubated in KRP at 37°C. At different time intervals, portions were removed and centrifuged at 3,000 g for 10 min at 4°C, and pellets and supernatants were counted for radioactivity.

ATP concentration in the PMN was measured by the luciferin-luciferase method (21). Glycogen concentration in the PMN was measured after alcohol precipitation as previously described (22). Activities of hexokinase, G6P, and 6-phosphogluconate dehydrogenases were measured as described previously (18). Protein concentration was determined by the Lowry method (23).

Statistical analysis

Data are presented as the mean \pm SD. For each assay, at least three observations were performed on separate occasions, unless otherwise indicated. The *t* test was used to compare the mean value of control assays with those of GSD Ib patients ($n = 3$). As only two GSD Ia patients were tested, no statistical analysis was performed.

Materials

D-[³H]2-Deoxyglucose and D-[¹⁴C]glucose were purchased from Amersham Corp. Radiochemical Centre, Amersham, England. All other chemicals were purchased from Sigma Chemical Co., St. Louis, MO.

Results

HMP shunt activity in intact PMN of GSD Ib patients (Fig. 1 B) was about one-third of that found in PMN of normal controls (Fig. 1 A); HMP activity in disrupted cells of GSD Ib

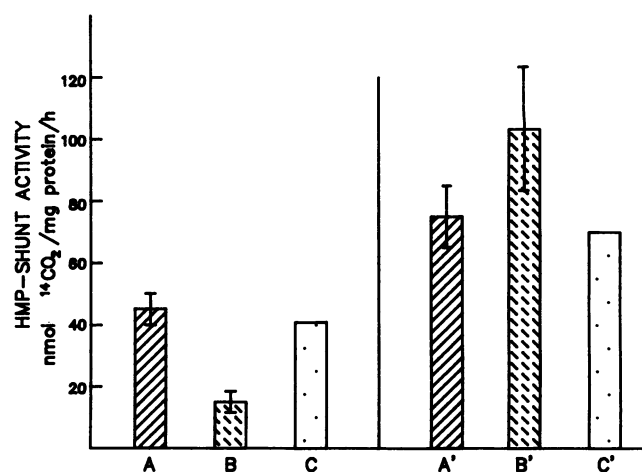


Figure 1. HMP shunt activity in intact and disrupted PMN of three GSD Ib patients and two GSD Ia patients as compared with controls. HMP shunt activity in intact and disrupted PMN was measured as described in Methods. A and A', intact and disrupted control PMN ($n = 12$); B and B', intact and disrupted GSD Ib PMN ($n = 3$); and C and C', intact and disrupted GSD Ia PMN ($n = 2$). The significant difference between A and B values was $P < 0.001$; between A and A' values, $P < 0.005$; between B and B' values, $P < 0.001$; and between A' and B' values, $P < 0.01$.

Table II. Activities of Glucose-6-Phosphate Dehydrogenase (G6PD), 6-Phosphogluconate Dehydrogenase (6PGD), and Hexokinase in PMN

	Control	GSDIb
	<i>nmol/10⁶ cells per min</i>	
Hexokinase	2.6±0.5	2.4; 3.0
G6PD	2.3±0.3	2.3; 1.7
6PGD	0.50±0.07	0.52; 0.74

Assays were carried out in duplicate on samples from five controls and two GSD Ib patients.

patients (Fig. 1 *B'*) was higher than in those from controls and one GSD Ia patient (Fig. 1, *A'* and *C'*). Table II shows that the activities of the rate-limiting enzymes of glycolysis and the HMP shunt—hexokinase, G6P, and 6-phosphogluconate dehydrogenase—were normal. During infections, HMP shunt activity in PMN of GSD Ib patients increased to the level found in cells from controls (Table III). HMP shunt activity in PMN of patients suffering from recurrent familial neutropenia was found to be within the normal range both in intact and in disrupted cells (Table III). In lymphocytes of GSD Ib, GSD Ia, and controls, HMP shunt activity was in the same range in both intact and disrupted cells (Fig. 2).

Glycolytic activity of PMN was determined by lactate production from glucose (Fig. 3). In intact cells of GSD Ib patients (Fig. 3 *B*), lactate production was 50% of that in controls (Fig. 3 *A*). In the absence of glucose lactate production from the endogenous substrate, there was no difference between control and GSD Ib patients (12±4 and 10.8 nmol/10⁶ per h, respectively). In disrupted PMN, glycolytic activity in all groups was similar. In lymphocytes, the glycolytic activity of both GSD Ia and GSD Ib patients did not differ from that in controls (data not shown).

As glycogen can serve as a substrate for lactic acid production, we measured glycogen concentrations in PMN of two GSD Ib patients. Glycogen concentrations in A.A.'s and L.S.'s PMN were found to be 20 and 28 mg/10⁶ cells, respectively, which is not significantly different from the glycogen concentration in control PMN: 25±7 mg/10⁶ cells.

The reduced activity of both HMP shunt and glycolysis in intact PMN of GSD Ib patients could have been due to a reduction either in glucose transport into the cells or in the availability of cofactors such as ATP, NAD, or NADP.

Table III. Activity of HMP Shunt in PMN of GSD Ib Patients during Infections

	HMP shunt activity	
	Intact	Disrupted
	<i>nmol CO₂/mg protein per h</i>	
Controls (15)	44±6	75±15
GSD Ib (3)	17±4	108±20
GSD Ib during infections (3)	39±5	78±10
Recurrent neutropenia (4)	46±6	90±15

Numbers of individuals examined are shown in parentheses.

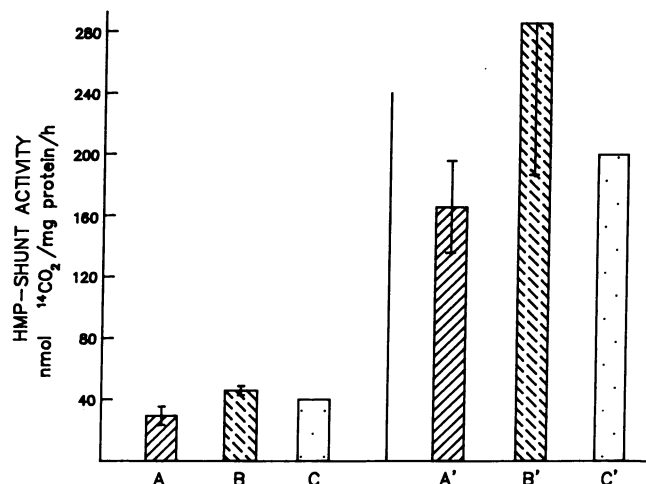


Figure 2. HMP shunt activity in intact and disrupted lymphocytes-monocytes of three GSD Ib patients and two GSD Ia patients as compared with controls. *A* and *A'*, intact and disrupted control lymphocytes-monocytes (*n* = 12); *B* and *B'*, intact and disrupted GSD Ib lymphocytes-monocytes (*n* = 3); and *C* and *C'*, intact and disrupted GSD Ia lymphocytes-monocytes (*n* = 2). There was no significant difference between *A* and *B* and between *A'* and *B'*. The significant difference between *A'* and *A* values was *P* < 0.001, and between *B'* and *B* values was *P* < 0.001.

Glucose transport into PMN was measured with [³H]2-deoxyglucose. Fig. 4 shows that the transport of 2-deoxyglucose into control PMN was linear with time up to 5 min, and was almost completely inhibited by 10 mM glucose or 0.1 mM phloretin, indicating that 2-deoxyglucose competes with glucose for entry into the cell. 2-Deoxyglucose transport into

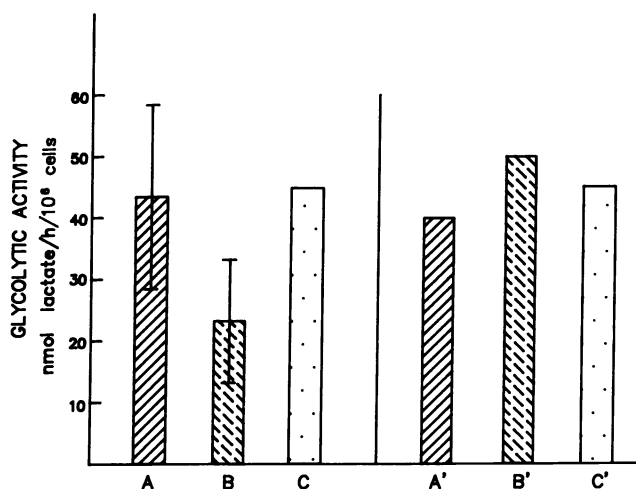


Figure 3. Glycolytic activity in intact and disrupted PMN of three GSD Ib patients and two GSD Ia patients as compared with controls. Glycolytic activity was measured by lactic acid production during 2 h of incubation with glucose as described in Methods. Lactic acid production was linear with time and with PMN concentrations *A* and *A'*, intact and disrupted control PMN (for *A*, *n* = 8, for *A'*, *n* = 3); *B* and *B'*, intact and disrupted GSD Ib PMN (for *B*, *n* = 3, for *B'*, *n* = 2); and *C* and *C'*, intact and disrupted GSD Ia PMN (*n* = 2). The significant difference between *A* and *B* values was *P* < 0.01.

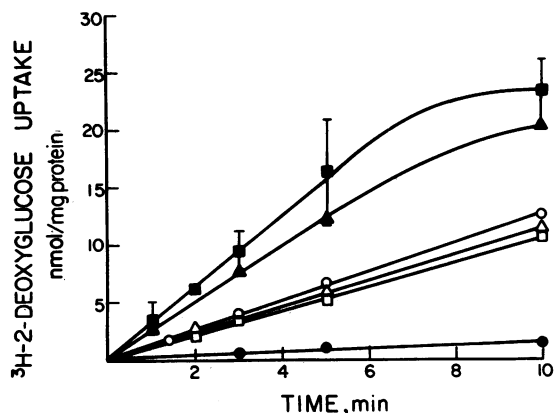


Figure 4. [^3H]2-Deoxyglucose uptake by PMN of three GSD Ib patients, one GSD Ia patient, and controls. [^3H]2-Deoxyglucose uptake was measured as described in Methods, at a final concentration of 2.5 mM [^3H]2-deoxyglucose. ■, control PMN ($n = 15$); ●, control PMN + 10 mM glucose; ▲, Ia patient's PMN; ○, Ib patient A.A.; △, Ib patient N.T.; and □, Ib patient L.S.

PMN of the three GSD Ib patients was only $\sim 30\%$ of that in controls or GSD Ia patients, and was also inhibited by 10 mM glucose (data not shown).

Transport of 2-deoxyglucose in normal PMN clearly followed saturation type kinetics and yielded K_m and V_{max} of 0.6 mmol/liter and 0.51 ± 0.13 nmol/min per 10^6 , respectively. A similar study performed in two of the patients showed a reduced V_{max} without a significant change in K_m (Fig. 5). The rate of deoxyglucose transport into PMN of a patient suffering from recurrent familial neutropenia (1,100 PMN cells/ μl) was found to be 0.47 nmol/ 10^6 per min, which is within the normal range.

The rate of phosphorylation of 2-deoxyglucose in the cells may affect glucose transport. As phosphorylation rates depend on ATP concentration, the level of ATP in PMN of GSD Ib patients was measured. Their ATP concentration was 0.57 ± 0.25 , as compared with 0.74 ± 0.31 nmol/ 10^6 cells in controls. The quantities of phosphorylated and nonphosphor-

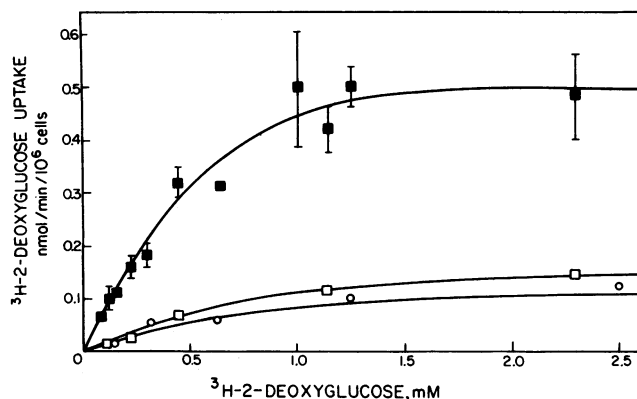


Figure 5. [^3H]2-Deoxyglucose uptake by PMN of controls and two GSD Ib patients at different concentrations. [^3H]2-Deoxyglucose uptake was measured as described in Methods at different substrate concentrations. K_m and V_{max} were calculated according to Lineweaver-Burk equation. ■, control PMN ($n = 8$); ○, Ib patient A.A.; and □, Ib patient L.S.

Table IV. Phosphorylation of [^3H]Deoxyglucose in PMN

Time of incubation <i>min</i>	Ratio of [^3H]deoxyglucose-6-P to [^3H]deoxyglucose	
	Control	L.S.
2	5.2	5.6
20	4.8	6

ylated deoxyglucose in the PMN of control and L.S.'s PMN were determined after 2 and 20 min of incubation with [^3H]2-deoxyglucose (Table IV). As can be seen, there was no significant difference in the phosphorylation of deoxyglucose between the PMN of the control and the patient.

To examine whether the cells were intact, we tested their resistance to various osmotic pressures. As can be seen in Fig. 6, the osmotic fragility of the PMN cells of the GSD Ib patients did not differ from that of the control.

Efflux of 2-deoxyglucose from loaded PMN was measured as described in Methods. The [^3H]2-deoxyglucose lost from the cells was fully accounted for by its appearance in the efflux medium. Its appearance in the supernatant increased in approximate proportion to time through at least 20 min of incubation. Calculations of the velocity of 2-deoxyglucose efflux were based on samples obtained between 0 and 20 min of incubation. The rate of efflux of GSD Ib PMN did not differ from those of control PMN (0.055 and 0.049 nmol/min per 10^6 , respectively).

In an additional experiment, uptakes of 2-deoxyglucose, lactate, and CO_2 production were assayed on PMN from the same blood sample. In accordance with previous results, 2-deoxyglucose transport into PMN of the patient was $\sim 30\%$ of that of the control. Glycolytic rates and HMP activity were similarly reduced in the PMN of GSD Ib patients; there was no difference, however, in the relative contribution of CO_2 and lactate production in cells from the patient and the control (Table V).

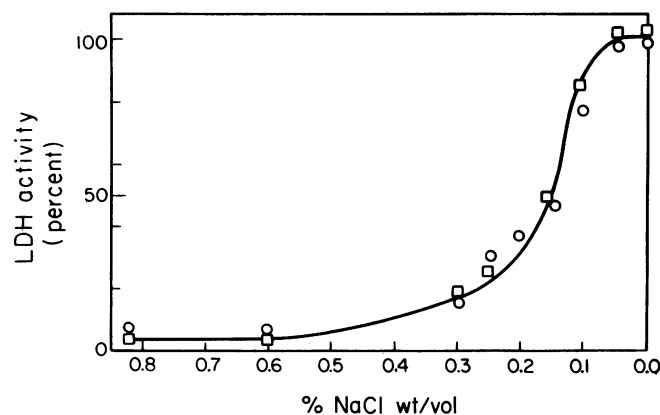


Figure 6. Osmotic fragility of PMN of GSD Ib patient and control. Osmotic fragility was measured as described in Methods. LDH activity in the supernatant after 30 min of incubation in water was considered as 100%. □, control; ○, patient A.A.

Table V. Distribution of Glucose Metabolites between Glycolysis and HMP Shunt

	GSD Ib	Control	GSD/control
	nmol/min per mg protein		%
Glucose transport	1.15 (100%)	3.70±0.4 (100%)	31.0
Lactate production	0.63 (55%)	2.00±0.2 (54%)	31.5
CO ₂ production	0.20 (17%)	0.70±0.06 (19%)	28.5
Lactate/CO ₂	3.1	2.8	

Glucose transport, lactate, and CO₂ production were measured as described in Methods in one patient and five controls on the same blood sample.

Discussion

The present study demonstrates an abnormal metabolism of PMN from patients with GSD Ib, manifested by a reduction in activity of the HMP shunt, glycolysis, and glucose transport into the cells. The reduced activities in PMN of GSD Ib patients could not be ascribed to differences in metabolic disturbances, such as recurrent hypoglycemia, lactic acidosis, or hyperuricemia, since glucose metabolism of PMN of GSD Ia patients did not differ from that of normal controls.

Our study confirms previous observations on abnormal HMP shunt activity in PMN of GSD Ib patients (11) and is in accordance with the results of Seger et al., who found a reduced oxygen burst during phagocytosis in whole cells, which was corrected by cell disruption and addition of NADPH (14). Seger (14) suggested that this reduced oxygen burst was due to a deficiency of NADPH in these cells. In the present study, NADPH levels were not measured, but low levels of NADPH cannot account for the reduced glycolytic activity; it is therefore unlikely that this could be the only defect in PMN from these patients. The present study shows that the metabolic defect in PMN of GSD Ib patients is not limited to the HMP shunt. The primary defect seems to be a reduction in the availability of G6P, causing a quantitatively similar impairment in both glycolytic and HMP shunt activity. The production of G6P in the cell depends on (a) glucose transport, (b) availability of ATP, (c) activity of hexokinase, and (d) degradation of glycogen. In the present study, hexokinase activity and ATP concentration in cells of GSD Ib patients did not differ from those of controls, leaving a reduced transport of glucose into the cell as the most likely alternative, which was confirmed by 2-deoxyglucose transport studies.

Part of 2-deoxyglucose accumulates in the neutrophils in a phosphorylated form; thus, the amount of 2-deoxyglucose retained by the neutrophils is a function of both the transport system and of the phosphorylation step.

As is evident from Table IV, there was no reduction in the ratio of 2-deoxyglucose-6-phosphate to 2-deoxyglucose in the patients' PMN as compared with control PMN either in zero *trans* conditions (2 min of incubation) or in steady state conditions (20 min of incubation). Thus, the rate of phosphorylation of 2-deoxyglucose is probably not the cause for the reduction in 2-deoxyglucose transport into the patients' cells observed at different substrate concentrations, as is evident in Fig. 5. However, one cannot exclude the possibility that the impairment of influx is secondary to a reduction in ATP con-

centrations in the presence of 2-deoxyglucose. Since glucose transport into the Ib neutrophils was diminished by 70%, the reduced production of C¹⁴O₂ through the HMP shunt could be due partly to the dilution of the radioactive G6P pool by cold G6P derived from endogenous glycogen. Yet such a dilution cannot explain the reduction in lactic acid production found in the patients' neutrophils (Fig. 3). The reason for the enhanced shunt activity found in the patients' neutrophils during infections (Table III) is not yet clear. It may be a result of activation of the HMP shunt, activation of glucose transport, or changes in the neutrophil population during infection.

The diminished rates of deoxyglucose uptake into the Ib PMN is probably not a reflection of a selected subpopulation of neutrophils, since transport of 2-deoxyglucose in PMN of a patient suffering from recurrent neutropenia was found to be within the normal range.

The diminished 2-deoxyglucose transport in the Ib PMN seems to be a specific and not a generalized defect in the membrane itself, since the osmotic fragility, which is a crude index of membrane integrity, did not differ from that of the controls (Fig. 6). Moreover, efflux of 2-deoxyglucose from loaded patient PMN was the same as in controls.

The relationship between the hepatic microsomal G6P translocase defect in the Ib patients and the neutrophil glucose transport defect is not clear. Roles of the multifunctional G6Pase system in insulin-independent glucose transport in various tissues have been suggested (24, 25). However, the possibility of a common subunit for glucose transport and microsomal G6P transport is unlikely, since in erythrocytes at least, the glucose transporter was found to be a single polypeptide (26).

A reduced transport of glucose into PMN can be due to a reduction in the number or activity of transporters. Synthesis of glucose transporters takes place in the endoplasmic reticulum (27) and may, in an unknown way, be directly dependent on the availability of microsomal G6P or indirectly on NADPH, a product of the microsomal hexose-6-phosphate-dehydrogenase (28, 29). A defect in the synthesis of glucose transporters could lead to a reduction in the number of transporters or to a change in properties, resulting in reduced transport capacity. The striking reduction in glucose transport observed in the PMNs of GSD Ib patients could be responsible for the metabolic and functional deficiencies in these cells.

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