AN INHIBITORY EFFECT OF D-MANNOHEPTULOSE ON LACTATE FORMATION IN HUMAN WHOLE BLOOD *IN VITRO*

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1. Introduction

A single dose of D-erythrose (300 mg) injected subcutaneously into rats has recently been found to produce a mild hyperglycemia in the injected animals [1]. Results of preliminary experiments did not exclude the possibility, among others, that the non-availability of active insulin in the circulation might be involved in the D-erythrose-induced hyperglycemia.

D-Mannoheptulose has been found to produce a transient hyperglycemia in rats [2], and to abolish the glucose-induced insulin release from pieces of pancreas [3, 4] as well as from isolated islets of Langerhans [5]. D-Mannoheptulose has been found to inhibit glucokinase and hexokinase of rat liver [6] and of other tissues (cited in [6]) and competitively inhibits the phosphorylation of glucose in homogenates of isolated islets of Langerhans [7]. These findings led to the suggestion that glucose phosphorylation in the β-cells may be a rate-determining step in the process of insulin release stimulated by glucose [6].

This suggestion also led us to examine the effect of D-erythrose on glycolysis. Human blood cells were chosen for this study. D-Erythrose has been found to have no effect on lactate production in blood cells *in vitro*. While no measurable effect of D-mannoheptulose on the ATP level and the rate of glucose disappearence from blood *in vitro* was found, the heptulose markedly inhibited lactate formation in the blood cells.

2. Materials and methods

Fresh blood was obtained from common blood-bank donors, by venipuncture with heparin as anticoagulant. Blood stored on acid-citrate-dextrose solution (U.S.P. formula A) was obtained from the Blood Bank of the Hadassah Medical School, and used within 3 weeks of storage. Hemoglobin-free hemolysates were obtained from cells of stored blood with the aid of diethylaminoethyl (DEAE) cellulose [8].

D-Mannoheptulose was the gift of Prof. E. Simon, The Weizmann Institute of Science, Rehovot, Israel. D-Erythrose was prepared from 2,4-*O*-ethylidene-D-erythrose [9] by acid hydrolysis, neutralization with barium carbonate, and careful deionization with a mixed-bed ion exchange resin. D-Erythrose was estimated by its oxidation with periodate and measurement of the formaldehyde formed with chromotropic acid [10].

Lactate [11] and pyruvate [12] were measured with lactic dehydrogenase. Glucose was estimated with glucose oxidase [13] and ATP with luciferase of fireflies' tails [14]. Hexokinase activity was measured with glucose-6-phosphate dehydrogenase [15] and expressed as μmole glucose-6-phosphate produced/mg protein/min. Protein was determined with the Folin phenol reagent [16].

Hexokinase was separated by vertical starch-gel electrophoresis [17] and detected by the fluorescence of $NADPH_2$ [18].

3. Results

Experiments with low D-erythrose concentration in whole blood (0.5 mM) and further experiments with high concentrations (15 mM, fig. 1) showed the tetrose to have no effect on blood cell lactate production *in vitro*. (D-Erythrose has also been found to have no effect on lactate production in rat whole blood *in vitro*).

D-Mannoheptulose (0.5 mM), on the other hand, showed a slight though significant inhibition of lactate production. Lactate formation in human fresh whole blood in the absence and in the presence of increasing added amounts of D-mannoheptulose (fig. 2) indicates that the heptulose exerts an inhibitory effect on lactate production in blood cells. This inhibition is proportional to the heptulose concentration and becomes prominent after the first 6 hr of incubation. No significant effect of D-mannoheptulose on pyruvate formation in blood cells was found. The rate of glucose disappearance from whole blood and the ATP level in the blood cells are not affected by the presence of the heptulose (fig. 3). The results presented in fig. 3 suggest that the delayed effect of the keto-heptose on lactate production in whole blood might be related to a slow passage of the heptulose into the cells, to the low accumulation of an active heptulose-derivative (either in the cells, or in the cell membrane), or to other factors.

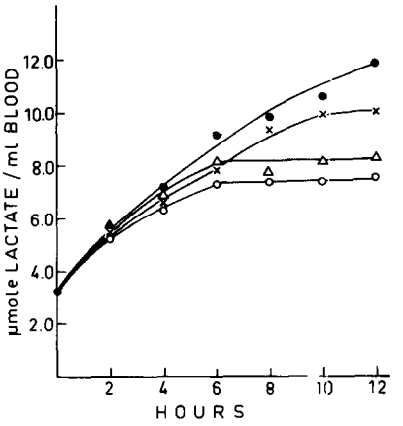

Fig. 2. Lactate production in human blood *in vitro* in the presence of added D-mannoheptulose. Isotonic D-mannoheptulose solution was added to whole blood to make the final concentration required. Final volumes of blood were adjusted with isotonic sucrose. D-Mannoheptulose concentration: None •; 0.5 mM ×; 5 mM △; 15 mM ○.

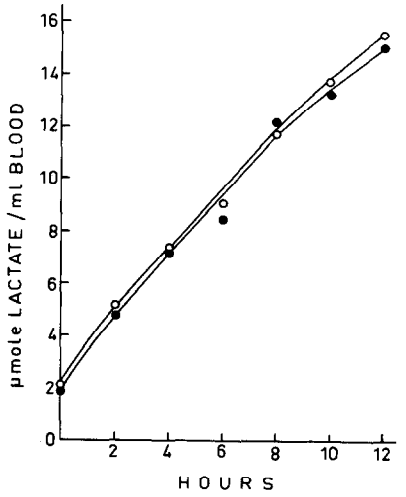

Fig. 1. Lactate production in human whole blood, *in vitro*, in the presence of added D-erythrose. Isotonic D-erythrose or sucrose solution was added to whole blood to make a final concentration of 15 mM. Blood with D-erythrose ○——○, with sucrose •——•.

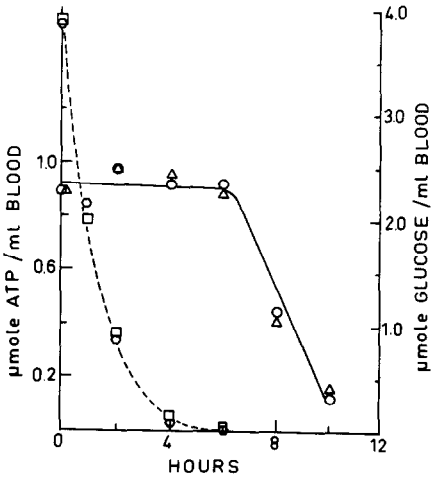

Fig. 3. Glucose and ATP content of whole blood in the presence and in the absence of added D-mannoheptulose. Blood made 15 mM with mannoheptulose △, or sucrose ○, ATP ——. Blood made 30 mM with D-mannoheptulose ◇, or sucrose □, glucose - - - -.

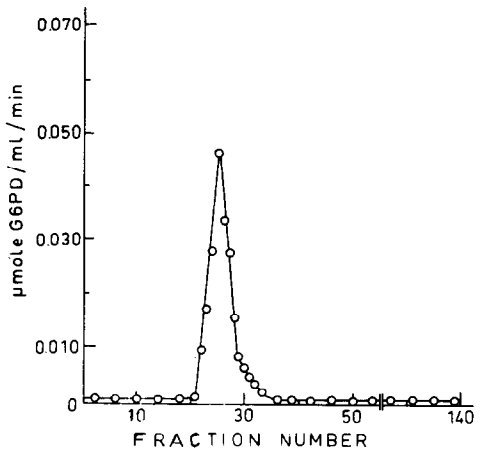

Fig. 4. Chromatographic separation of blood cell hexokinase on DEAE cellulose. Chromatography was performed at 4° at a rate of 40 ml/hr. Fractions of 14 ml were collected. The enzyme in the eluant was reprecipitated with ammonium sulfate and dialysed against 0.01 mM phosphate buffer.

Blood cell hexokinase was purified as follows. Hexokinase of hemoglobin-free hemolysates [8] was separated at 40–55% ammonium sulfate saturation at 4°. The precipitate was dissolved in 0.01 M phosphate buffer pH 7.0, containing EDTA-Na_2 (5 mM), β-mercaptoethanol (5 mM) and glucose (10 mM), and dialysed against the same buffer. The enzyme was adsorbed on a DEAE cellulose column and eluted with a linear 0.0–0.6 M potassium chloride (2L) gradient. The enzyme came off the column as a single fraction (fig. 4) at 0.09–0.14 M potassium chloride.

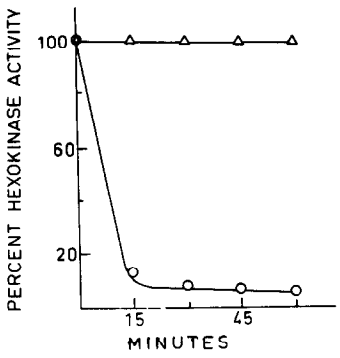

Fig. 5. Blood cell hexokinase activity at 45°. Hexokinase in 100 mM glucose △——△; in 10 mM glucose ○——○.

Table 1
Blood cell hexokinase activity in the presence of 15 mM mannoheptulose.

ATP mM	μmole G6P formed/10 min		Inhibition (%)
	MH	+MH	
5.0	0.710	0.630	11.0
2.5	0.690	0.625	9.5
1.25	0.510	0.460	9.5
0.62	0.370	0.240	35.0
0.31	0.144	0.048	67.0
0.16	0.067	0.019	71.0

Reaction mixture (1.0 ml): phosphate 0.7 mM (pH 7.6); glucose, 25 mM; D-mannoheptulose 15 mM; NADP, 0.25 mM; ATP, see the table; $MgCl_2$, 5.0 mM; mercaptoethanol, 5.0 mM; G-6-P dehydrogenase 0.3 μ; hexokinase, 0.1 ml.

By this stage the enzyme had been purified 6900-fold.

The hexokinase of blood cells separated on DEAE cellulose has a pH optimum of 7.0–8.5, an electrophoretic migration in starch gel as that of hexokinase II of rat liver [17], and like this enzyme is inactivated in 10 mM glucose at 45°, whereas it is stable under the same conditions in 100 mM glucose (fig. 5). The blood enzyme has an apparent K_m for glucose of 0.24 mM and for ATP of 0.76 mM.

The enzymatic activity of blood cell hexokinase in 0.7 mM phosphate buffer, 25 mM glucose, in the absence and in the presence of varying ATP concentrations (table 1), indicates that at 15 mM mannoheptulose the inhibitory effect of the heptulose is inversely proportional to the ATP concentration. At 0.15–0.3 mM ATP, hexokinase activity is about 70% inhibited.

D-Erythrose was found to have practically no effect on blood cell or yeast (Boehringer) hexokinase activity.

4. Discussion

Previous work showed the inhibitory effect of D-mannoheptulose on glucose phosphorylation in homogenized islets of Langerhans to be inversely proportional to glucose concentration [7]. Extracts of mouse islets did not show evidence for the presence of glucokinase and 'it appears unlikely therefore that

glucokinase is responsible for the increased rate of glucose oxidation and insulin release' [19]. Glucose oxidation, like release of insulin, was strongly inhibited by mannoheptulose but not by phlorhizin. It has later also been suggested that 'the rate of glucose oxidation in islets may be influenced by factors other than the rate of glucose phosphorylation' [20].

D-Mannoheptulose has been shown to inhibit lactate formation in blood cells (fig. 2) with no measurable concomitant change in the rate of glucose disappearance from the blood (fig. 3), in accordance with the finding that the inhibitory effect of mannoheptulose on blood cell hexokinase is inversely proportional to the ATP concentration. It also accords with the fact that no change of the ATP content of the cells is measurable before the glucose level of these cells reaches the limit of analysis (fig. 3). These results indicate that mannoheptulose may inhibit glycolysis at a stage beyond the phosphorylation of glucose.

The pentose phosphate shunt is operative in the blood cells [21]. The possibility that mannoheptulose partly effects lactate production by modifying this metabolic pathway cannot be excluded.

The finding that D-erythrose has no effect on lactate production in human blood suggests, to the extent that deductions can be made from the metabolism of human (and rat) blood cells *in vitro* to the intact rat, that glycolysis seems not to be involved in the D-erythrose-induced hyperglycemia in the rat.

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