The Variation of Glucose Metabolism in Human Erythrocytes in the Presence of L-Thyroxine[1] (35819)

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(Introduced by R. A. MacDonald)

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The metabolism of 2,3-diphosphoglyceric acid (2,3-DPG) in the mature erythrocyte is regulated by a dynamic balance among the enzymes and substrates of the phosphoglycerate cycle. Recently, thyroid hormones have been shown to stimulate, 2,3-DPG synthesis in intact red cells (1, 2) and in an hemoglobin-free and enzyme-rich preparation, utilizing 1,3-DPG as substrate (1). The present report was designed to study the effect of L-thyroxine (T_4) on glucose metabolism of human intact erythrocytes.

Materials and Methods. Fresh venous blood was drawn from normal human subjects in heparinized vacutainer tubes, and the plasma and buffy coat were removed by centrifugation at 1000*g* for 10 min. The red cells were washed 3 times in equal volumes of Krebs–Ringer bicarbonate buffer (pH 7.5) and adjusted to a 40% hematocrit in a solution (pH 7.5–7.6) containing 66% Krebs–Ringer bicarbonate and 34% deproteinized plasma (3) enriched with 10 m*M* glucose and 3 m*M* inorganic phosphate. Four milliliter aliquots of red cell suspension with, or without, T_4 (3.75×10^{-8} *M*) were transferred to Erlenmeyer flasks and 0.5 μCi of glucose-6-^{14}C were added. The flasks were sealed, and gassed for 30 sec with 5% CO_2 in air, and incubated at 37° in a metabolic shaker at 70 oscillations/min for varying periods of time. Samples were collected and deproteinized in 0.5 vol of cold perchloric acid, 6% (w/v), following determination of pH, hemoglobin, and hematocrit. The extract was kept at least 15 min on ice, spun at 12,000*g* for 10 min; and the clear supernatant was neutralized (pH 7.0) with 1 *N* potassium hydroxide. The neutralized extracts were assayed for glucose (4), lactate (5), ATP, ADP (6), fructose 1,6-diphosphate, and dihydroxyacetone (7); 2,3-DPG was determined enzymatically (8). The $^{14}CO_2$ and the percentage of glucose metabolized through the hexose monophosphate (HMP) shunt were estimated by the method described by Murphy (9).

Results. The metabolic activity of T_4 on glucose metabolism was studied in 10 experiments done in duplicate. The cells incubated with T_4 showed, during the first 30 min of incubation: increased glucose consumption, slight depression of lactate production, and increased 2,3-DPG production (Table I). However, incubation beyond 30 min failed to show further increments of metabolic changes (Table I). Moreover, the absolute value of 2,3-DPG in the samples containing T_4 remained elevated above the control levels (Fig. 1).

On the contrary, ATP and ADP levels fell in the T_4-treated cells (Table II) within the first 30 min of incubation and during the following 2 hr, but returned to control levels by the fourth hour. Fructose 1,6-diphosphate and dihydroxyacetone phosphate levels rose along with increasing incubation time in both test and control cells (Table I). The HMP shunt activity did not show significant variation; 5.51% ± 0.82 SD/hr for control and 5.31% ± 0.74 SD/hr for the T_4-treated cells.

Discussion. The metabolic variations of control erythrocytes incubated at pH 7.55–7.6 at 37° are secondary to an increased enzymatic activity of the phosphofructokinase

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TABLE I. Effect of T_4 (3.75×10^{-8} *M*) on Glucose Metabolism of Erythrocytes.[a]

Time (min):		(μmoles/ml cells $\pm$ SD) 30	60	120	180	220
Glucose utilized	Control	0.92 $\pm$ 0.16	0.99 $\pm$ 0.11	2.4 $\pm$ 0.16	2.0 $\pm$ 0.14	1.6 $\pm$ 0.27
	Test	1.4 $\pm$ 0.13	0.90 $\pm$ 0.09	2.4 $\pm$ 0.24	1.9 $\pm$ 0.17	1.7 $\pm$ 0.31
Lactate formed	Control	1.71 $\pm$ 0.16	1.23 $\pm$ 0.18	3.47 $\pm$ 0.43	2.22 $\pm$ 0.13	1.11 $\pm$ 0.31
	Test	1.65 $\pm$ 0.21	1.23 $\pm$ 0.16	3.38 $\pm$ 0.62	1.88 $\pm$ 0.21	1.77 $\pm$ 0.26
2,3-DPG formed	Control	0	0	0	0	0
	Test	1.5 $\pm$ 0.16	0	0	0	0
Fructose diphosphate	Control	0.151 $\pm$ 0.011	0.171 $\pm$ 0.011	0.182 $\pm$ 0.005		0.500 $\pm$ 0.024
	Test	0.140 $\pm$ 0.003	0.168 $\pm$ 0.013	0.194 $\pm$ 0.010		0.580 $\pm$ 0.026
Dihydroxy-acetone	Control	0.014 $\pm$ 0.006	0.27 $\pm$ 0.009	0.35 $\pm$ 0.015		0.650 $\pm$ 0.032
	Test	0.010 $\pm$ 0.008	0.19 $\pm$ 0.008	0.29 $\pm$ 0.013		0.620 $\pm$ 0.042

[a] The washed red cells were suspended in 66% Krebs–Ringer bicarbonate buffer and 34% normal deproteinized plasma, final pH 7.5–7.6. The entire suspension was gassed for 30 sec with 5% CO_2 in air and incubated at 37° in a metabolic shaker.

step (10, 11). Under these circumstances the limiting reaction of the glycolytic pathway shifts from phosphofructokinase to glyceraldehydephosphate dehydrogenase reaction (10, 12). However, when phosphorus is added, the lack of NAD as an hydrogen acceptor acts as the limiting factor (13), due to a decrease in the proportion of pyruvate available to act as an oxidant (14). Subsequently, NADH formed during the glyceraldehydephosphate dehydrogenase reaction is not converted to NAD by lactate dehydrogenase, and the NADH/NAD ratio increases. This leads to an inhibition of the glycolytic pathway at the level of glyceraldehydephosphate dehydrogenase (15) with accumulation of intermediates such as fructose 1,6-diphosphate and dihydroxyacetone phosphate (Table I). Since ATP synthesis follows the glyceraldehydephosphate dehydrogenase step, an excess of ATP utilization over ATP formation occurs, as indicated by an increased ADP/ATP ratio (Table II). The elevated ADP/ATP ratio activates the phosphofructokinase with further accumulation of higher intermediates. This sequence of events explains the decreased glucose consumption, 2,3-DPG and lactate production

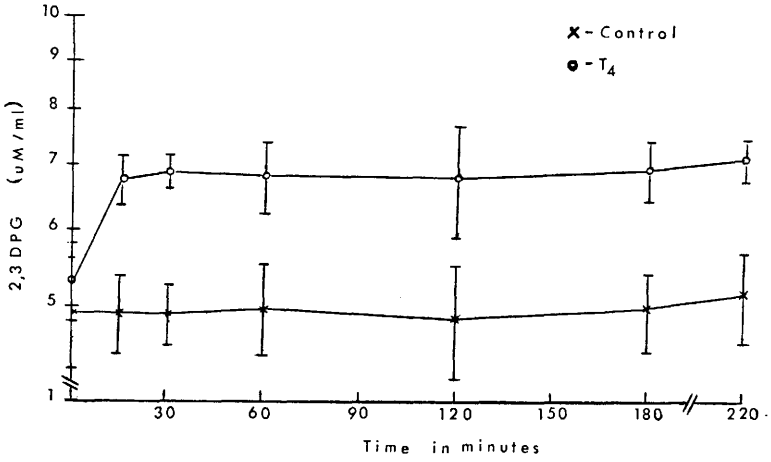

FIG. 1. 2,3-DPG values (μmoles/ml $\pm$ SD) of control and test (T_4 3.75×10^{-8} *M*) erythrocytes.

TABLE II. Effect of T_4 (3.75×10^{-8} *M*) on RBC Nucleotides.[a]

Time (min):		(μmoles/ml of cells ± SD) 0	30	120	180	220
ATP	Control	1.78 ± 0.054	1.84 ± 0.090	1.54 ± 0.061	1.50 ± 0.062	1.53 ± 0.058
	Test	1.71 ± 0.050	1.65 ± 0.072	1.38 ± 0.047	1.38 ± 0.051	1.56 ± 0.047
ADP	Control	0.121 ± 0.040	0.115 ± 0.06	0.116 ± 0.06	0.130 ± 0.07	0.120 ± 0.06
	Test	0.120 ± 0.060	0.111 ± 0.05	0.106 ± 0.04	0.110 ± 0.07	0.125 ± 0.09
ADP/ATP	Control	0.067	0.064	0.0753	0.086	0.0784
	Test	0.071	0.067	0.0770	0.080	0.810

[a] The cells were suspended in 66% Krebs–Ringer bicarbonate buffer and 34% normal deproteinized plasma with final pH 7.5–7.6, gassed for 30 sec with 5% CO_2 in air, and incubated at 37° in a metabolic shaker.

during continued incubation in our experiments (Table I).

The thyroid hormone effect on intact erythrocytes in our experiments apparently shifts glucose through the phosphoglycerate cycle rather than through the pathways described above. Subsequently, increase in 2,-3-DPG and glucose consumption occurs while lactate production and ADP/ATP ratio decrease (Tables I, II). This phenomenon is complete within 30 min, but 2,3-DPG remains elevated (Fig. 1).

As recently demonstrated (1), T_4 affects diphosphoglycerate mutase either by direct stimulation and/or alteration of the inhibition constant (K_i). Rose (14) has shown that the K_i of purified diphosphoglycerate mutase is extremely low (0.85 μM), thus thyroid hormone could play an important modifying role, allowing the erythrocyte to continue synthesis of 2,3-DPG, above physiologic levels.

Summary. Normal washed human erythrocytes, when incubated in a buffered saline solution (pH 7.5–7.6) with T_4 (3.75×10^{-8} *M*), demonstrated increased production of 2, 3-DPG and glucose consumption during the first 30 min of incubation. However, lactate production and ATP levels were decreased. The mechanism is probably related to a direct effect of T_4 on the diphosphoglycerate mutase.

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1. Snyder, L. M., and Reddy, W. J., J. Clin. Invest. **49**, 1993 (1970).

2. Miller, L. D., Sugerman, H. J., Miller, W. W., Delivoria-Papadopoulos, M., Diaco, J. F., Gottlieb, A. J., and Oski, F., Ann. Surg. **172**, 1051 (1970).

3. Ermans, A. M., and Galperin, H., *in* "Progress in Endocrinology" (K. Forherby, J. A. Loraine, J. A. Strong, and P. Eckstein, eds.), p. 124. Cambridge Univ. Press, London (1960).

4. Hultman, E., Nature (London) **182**, 108 (1959).

5. Hohorst, H. J., *in* "Methods of Enzymatic Analysis" (H. U. Bergmeyer, ed.), p. 267. Verlag Chemie, Weinheim/Bergstresse, Academic Press, New York (1965).

6. Bucher, T., and Hohorst, H. J., *in* "Methods of Enzymatic Analysis" (H. U. Bergmeyer, ed.), p. 246. Verlag Chemie, Weinheim/Bergstrasse, Academic Press, New York (1965).

7. Strehler, B. L., *in* "Methods of Enzymatic Analysis" (H. U. Bergmeyer, ed.), p. 599. Verlag Chemie, Weinheim/Bergstrasse, Academic Press, New York (1965).

8. Beautler, E., Meul, A., and Wood, L. A., Transfusion (Philadelphia) **9**, 109 (1969).

9. Murphy, J. R., J. Lab. Clin. Med. **55**, 286 (1960).

10. Minakami, S., Saito, T., Suzuki, C., and Yoshikawa, H., Biochem. Biophys. Res. Commun. **17**, 748 (1964).

11. Rizzo, S., and Eckel, R. E., Amer. J. Physiol. **211**, 429 (1966).

12. Mills, G. C., Arch. Biochem. Biophys. **117**, 487 (1966).

13. Reinauer, H., and Bruns, F. H., Biochem. Z. **340**, 503 (1964).

14. Rose, I. A., and Warms, V. B., J. Biol. Chem. **241**, 4848 (1966).

15. Furfine, C. S., and Welick, S. F., J. Biol. Chem. **240**, 844 (1965).

16. Rose, Z. B., J. Biol. Chem. **243**, 4810 (1968).

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