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The Uptake of 3-Deoxy-3-Iodine-Glucose by Normal and Tumor cells *In Vitro*

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It is found that 3-deoxy-3-iodine-glucose (3D-I-glucose) enters both erythrocytes and tumor cells at a rate close to that of glucose entry. For tumor cells the total uptake of the preparation was 6-fold higher than for erythrocytes (1500 and 250 μM/mln. cells, respectively). Phosphorylated products of 3D-I-glucose were not detected; however, the total amount of preparation in the probes dropped during incubation, indicating that it is metabolized by the cells.

Key Words: *3D-I-oxyglucose; human erythrocytes; tumor cells*

The metabolism of saccharide derivatives is important and interesting from the viewpoint of applied problems of diagnostics and treatment of malignant neoplasms [1,3,4]. At the present time modified saccharides, for example, 2-fluorine-D-glucose, have found application not only in experimental oncology but also in clinical practice [5,6]. At the same time, the metabolic peculiarities of the majority of saccharides have so far not been studied. It should be noted that, theoretically, iodine derivatives of glucose may carry both a diagnostic marker and therapeutic activity (^{125}I and ^{131}I) to tumor tissues.

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In the present work we studied the uptake of 3-deoxy-3-iodine-glucose by normal and tumor cells *in vitro*.

MATERIALS AND METHODS

3D-I-glucose was synthesized at the Zelinskii Institute of Organic Chemistry, Russian Academy of Sciences. Its stability was checked by NMR spectroscopy. The concentration of 3D-I-glucose was measured by the orthotoluidine method [2]; the sensitivity of this method to the preparation is somewhat higher than to glucose. Since the method is sensitive to all reduced saccharides, the contribution of glucose was determined in an Eksan-G analyzer (PZTM, Lithuania) based on the principle of a glucose oxidase electrode (3D-I-glucose cannot be

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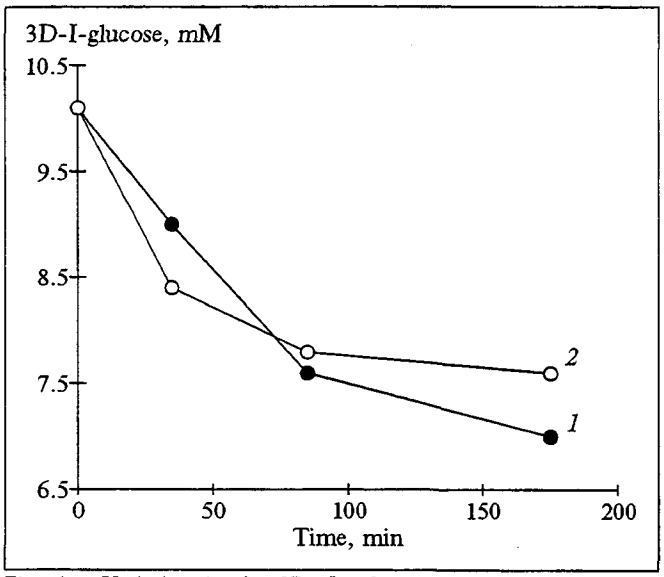

Fig. 1. Variation in the 3D-I-glucose concentration in the supernatant (*1*) and lysate (*2*) of an erythrocyte suspension (4 mln. cells/ml).

detected by the glucose oxidase method). The entry of 3D-I-glucose in the cells was studied in suspensions of erythrocytes and tumor cells. Fresh preparations of human erythrocytes were used in the experiments. Tumor cells were derived from the murine ascitic sarcoma S37 and cervical cancer (CC5) passaged on CBA and CBWA mice, respectively (Oncology Research Center, Russian Academy of Medical Sciences). Human erythrocytes and ascitic cells were washed three times in Ringer's solution and resuspended in it. The cell concentration was determined in a Goryaev chamber. Cell viability was not monitored. For a comparative analysis of 3D-I-glucose entry in the cells parallel measurements were performed with glucose and glucose-3D-I-glucose mixtures in the same cell suspensions. The latter also served as an arbitrary control of cell viability. The suspensions were incubated at 37°C with constant stirring, and aliquots with a known amount of 3D-I-glucose in Ringer's solution were added to the incubation medium, and the suspension aliquots were then withdrawn at fixed time intervals for analysis. The probes were either treated with trichloroacetic acid or centrifuged immediately, and the concentration of 3D-I-glucose was measured in the supernatant and the pellet lysate. The phosphorylation of 3D-I-glucose was studied in a medium containing 1.1 ml 0.05 M Tris-HCl buffer (pH 7.4), 6.8 mg ATP, 0.11 ml 100 mM $MgSO_4$, and 5.1 αl 40% glucose (or 4.4 mg 3D-I-glucose). Phosphorylated products were identified by thin-layer chromatography [7].

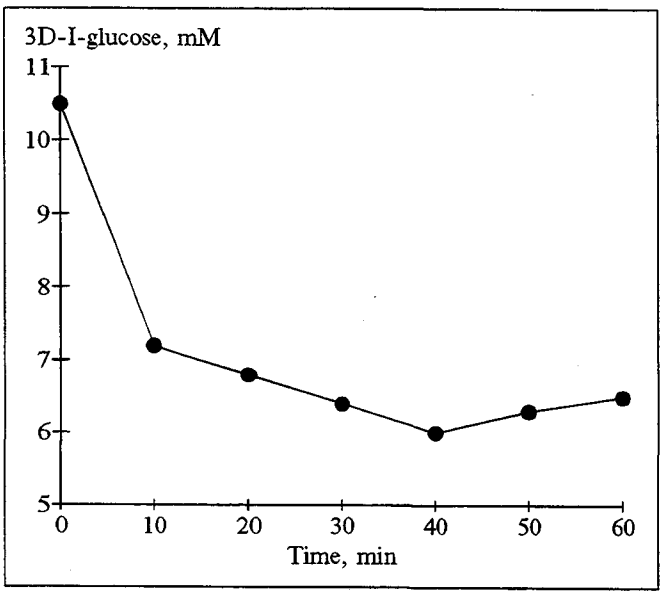

Fig. 2. Variation in the 3D-I-glucose concentration in the medium upon incubation with CC5 cells (2 mln. cells/ml).

RESULTS

Figure 1 illustrates changes in the 3D-I-glucose concentration in the incubation medium for erythrocytes. The 3D-I-glucose concentration in the supernatant changed nonlinearly; it decreased 30% after 3 h of incubation, and only about 30% of the initial 3D-I-glucose concentration was detected in the supernatant after 24 h of incubation. However, in the lysate of the cell suspension the concentration of 3D-I-glucose was higher than in the supernatant after 1.5 h of incubation, implying the accumulation of 3D-I-glucose (or its products detected by the o-toluidine method) in erythrocytes. The difference between the 3D-I-glucose concentration in supernatant and lysate increased with time; however, the total concentration of the preparation decreased, testifying to chemical transformations of 3D-I-glucose in the cell; the products of these transformations cannot be determined by the o-toluidine method.

Less than 30% of the initial concentration of 3D-I-glucose remained in the suspension after 24 h of incubation. The rate at which 3D-I-glucose was taken up from the medium was practically the same as the rate of glucose uptake at the same initial concentration. There was a tendency toward a greater uptake of glucose after the addition of equal concentrations of 3D-I-glucose and glucose (6 mM) to the incubation medium.

The metabolic pathways for 3D-I-glucose remain unclear, since we did not detect phosphorylated products either after incubation of 3D-I-glucose with hexokinase or in a homogenate of cells preincubated with 3D-I-glucose.

Upon incubation of 3D-I-glucose with tumor cells (CC5) its concentration in the incubation medium diminished faster then upon incubation with erythrocytes. A tendency toward a decrease in the uptake rate was observed (Fig. 2). The total uptake of 3D-I-glucose by the tumor cells was 6-fold higher than the uptake by erythrocytes (1500 and 250 μM/mln. cells, respectively). A similar dynamics of concentration changes was observed upon incubation of 3D-I-glucose with S37 cells. Figure 3 shows the rate of 3D-I-glucose accumulation in S37 cells. It should be mentioned that the dynamics of concentration changes in cells and supernatant correlate between themselves. When glucose and 3D-I-glucose were added to the cell suspension in an equimolar concentration, the rate of 3D-I-glucose uptake did not change, indicating that the uptake of 3D-I-glucose was not inhibited by glucose at the studied concentrations.

Thus, 3D-I-glucose enters both normal and tumor cells *in vitro*. Its accumulation in tumor cells S37 and CC5 proved to be considerably higher than in normal erythrocytes. The results obtained indicate that further *in vivo* studies of 3D-I-glucose as a potential carrier of a diagnostic marker or therapeutic activity are justified.

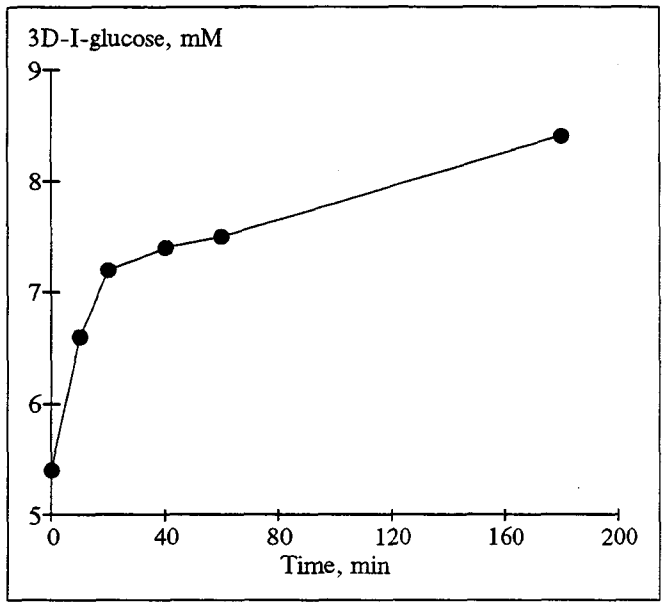

Fig. 3. Accumulation of 3D−I−glucose in murine sarcoma S37 cells. The cell concentration in the suspension is 2.8 mln./ml.

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