ACTIVATION OF GLYCOLYSIS IN TUMOR TISSUE BY INORGANIC PHOSPHATE AT LOW pH VALUES

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Experiments in vitro showed that the activating action of inorganic phosphate (P_i) on glucose utilization by tumor tissue continues to be observed at low pH values of the incubation medium. Addition of 0.15 M Na_2HPO_4 solution to Tris-HCl buffer at the time when self-acidification of the tumor ends during glucose infusion, led to a further decrease in pH of the tumor tissue. pH values of the tumor of the order of 4.5-4.6 could be obtained. The use of solutions containing P_i to acidify tumors during optimization of combined treatment schemes appears promising.

KEY WORDS: tumor; pH; glycolysis; inorganic phosphate; acidification.

One of the most important components of the "multistage treatment of cancer" and developed by von Ardenne [1] is selective self-acidification of the tumor tissue. A decrease in pH is essential in order to activate the lysosomal enzymes which destroy tumor cells and to optimize the action of alkylating cytostatics. Self-acidification is produced by injecting glucose solution into the tumor carrier. The lowest pH of tumor tissue which has been obtained experimentally, when nicotinamide-adenine nucleotide was injected along with glucose when self-acidification ceased, was 5.45 [2]. The cessation of acidification is explained primarily by the fact that the hexokinase activity of tumor tissue is sharply inhibited in an acid medium [3, 6, 8]. In an attempt to obtain as low pH values as possible, which would be optimal for both chemotherapy and radiotherapy [1, 7], it was decided to make use of data [5] showing the activating action of inorganic phosphate (P_i) on glycolysis in tumor cells. However, doubts were aroused by the fact that in the paper cited [5] the activation effect was obtained in an incubation medium at pH 7.4, whereas in our own experiments the pH of the tumor at the moment of suggested addition of P_i was usually 5.5-5.6.

In this investigation activation of glycolysis of tumor tissue by P_i was studied at low pH values.

EXPERIMENTAL METHOD AND RESULTS

Experiments were carried out on rats with a subcutaneously transplanted Guerin's carcinoma, the tissue of which was minced and a homogenate prepared in physiological saline (1 : 10), 0.5 ml of which was incubated in 2 ml of medium containing 12 μM ATP, 8.3 μM glucose, and 60 μM KF. The concentration of P_i was $1 \cdot 10^{-3}$ M. The pH was stabilized with 0.15 M Tris-maleate buffer. The choice of values of pH of the incubation medium was determined by the fact that on addition of glucose the pH range in the tumor tissue was 5.0-7.0. The glucose concentration in the medium was determined by an enzymic method [4]. The action of P_i on the course of self-acidification was studied in experiments in which a 20% solution of glucose was injected intravenously into the rats at the rate of 80 mg/kg/min, together with a solution of P_i, with simultaneous recording of the pH of the tumor by means of a glass microelectrode; the indices were recorded on graph paper by a KSP-4 automatic writer (the technical aspects of determination of pH were dealt with by the engineer M. V. Sidorenko). The solution containing P_i for injection into the animals consisted of 0.15 M Na_2HPO_4 solution stabilized with 0.15 M Tris-HCl buffer, with an equal volume of 40% glucose solution, and its pH was 7.35. Injection began when the pH of the tumor, having reached a certain value during infusion of pure glucose, ceased to fall, as was usually observed after 92.0 ± 8.8 min. The results are given in Figs. 1 and 2.

A reduction in hexokinase activity with a decrease in pH of the medium and the activating effects of P_i at pH 6.9 are in agreement with existing data [3, 5, 6, 8]. A new fact was found, namely that despite the decrease

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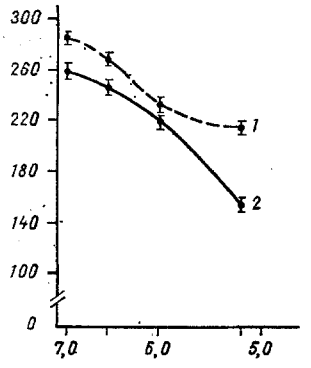

Fig. 1

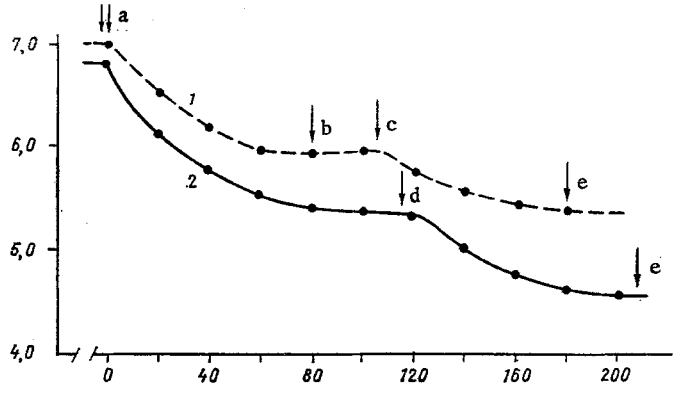

Fig. 2

Fig. 1. Utilization of glucose by tumor tissue in incubation medium at different pH values. Abscissa, pH; ordinate, quantity of glucose (in μmoles) utilized per gram tissue per hour. 1) In presence of P_i, 2) without P_i.

Fig. 2. Value of pH of tumor tissue during administration of solutions of glucose (a), glucose plus Tris-HCl buffer (b), glucose plus Tris-HCl buffer plus P_i (c), glucose plus P_i (d), and at end of injection (e). Abscissa, duration of administration (in min); ordinate, pH. 1) Experiment on 2/17/76; 2) experiment on 12/17/76.

in pH the activating effect of P_i remained, and the decrease in hexokinase activity which nevertheless took place was less marked than in the absence of P_i. Activation of glucose utilization was observed both when a 0.15 M solution of K_2HPO_4 was used (Fig. 1) and also in the presence of a 0.15 M solution of Na_2HPO_4 (at pH 6.9 without P_i the rate was 253.6 ± 12.8 μmoles glucose/h/g tissue, with P_i it was 272.8 ± 9.6; at pH 5.2 the rate without P_i was 136.0 ± 10.9 and with P_i 206.9 ± 10.6 μmoles/h/g tissue). This indicates that it was the P_i ions which had an activating action on glucose utilization by the tumor tissue homogenate and, moreover, at low pH values. The next step was to verify this effect of P_i on glycolysis of tumor tissue in experiments in vivo.

It was found that addition of P_i led to renewal of the fall of pH which usually was stabilized at 5.35 ± 0.07 level (initial value 6.70 ± 0.04) and led to considerable self-acidification of the tumor tissue, as shown by a mean pH within the tumor of 4.90 ± 0.09. The effect of P_i began to be manifested 12.8 ± 3.8 min after the beginning of administration of glucose solutions with P_i, and it continued on average for 66.9 ± 6.7 min. In some experiments pH values of the order of 4.5-4.6 in the tumor could be obtained. After injection of glucose with Tris-HCl buffer but without P_i solution no increase in self-acidification took place, but the addition of P_i to the injected mixture caused a further decrease in pH (Fig. 2). This proves the decisive role of P_i in the activation of glycolysis and the reduction in pH of tumor tissue during glucose administration.

The results suggest that tumor tissue can respond to the action of P_i by activation of glycolysis not only at physiological, but also at low pH values, and both in vitro and in vivo. The activation of glycolysis of tumor tissue by P_i probably correlates closely with its hydrogen ion concentration, an increase of which up to a certain level potentiates the activating action of P_i on glucose utilization by tumor cells. The use of solutions containing P_i to obtain maximal acidification of tumor tissue in order to optimize certain schemes for combined treatment of tumors would appear to be promising.

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