ACCUMULATION OF INTRAMUSCULARLY INJECTED FOREIGN ENZYMES BY EHRLICH'S CARCINOMA CELLS

F. B. Levin, T. T. Berezov, and V. A. Gusev

UDC 616.006.6-092.008:931

Glucose oxidase or an extract of rat liver containing tyrosine aminotransferase and glucose-6-phosphatase was injected intraperitoneally into mice with an Ehrlich's ascites carcinoma. High activity of these foreign enzymes was detected biochemically and histochemically in the washed tumor cells and is evidence of their accumulation by cancer cells.

Now that enzymes are used in the treatment of tumors it is important to study the mechanisms of action of enzyme preparations and the ways in which they exert their action on neoplastic tissues. Investigations have shown that high-molecular-weight biopolymers (nucleic acids, polypeptides, proteins) can penetrate inside the tumor cells [2, 3, 5]. However, the interaction between foreign enzymes and tumor cells has not yet been adequately investigated.

The investigation described below was undertaken to study the accumulation of enzymes, injected intraperitoneally into mice with an Ehrlich's carcinoma, by the tumor cells.

EXPERIMENTAL METHOD

Hydrocortisone was injected into a rat in a dose of 10 mg/100 g body weight and an extract subsequently prepared from the animal's liver was used as the source of the enzymes tyrosine aminotransferase and glucose-6-phosphatase. Part of the liver tissue was homogenized in the cold with three parts physiological saline and centrifuged for 40 min at 8000 rpm. The supernatant was used in the experiments. A crystalline commercial preparation of glucose oxidase (L'vov factory) was used in a concentration of 5-15 mg/ml physiological saline.

Mice with an ascites tumor of 6-7 days' duration (with appreciable enlargement of the abdomen) received an intraperitoneal injection of 3 ml rat liver extract or 1 ml of a solution of glucose oxidase in physiological saline, warmed to 37°C. The puncture wound was sealed with collodion and in 3 h the animals were killed. The cell suspension was filtered through two layers of gauze and washed five times with 50 volumes cold physiological saline, with centrifugation for 10 min at 800 rpm. The activity of the foreign enzymes, previously added to the medium, was determined quantitatively in the washed cells. Absence of enzyme activity in the last portion of the physiological saline used for washing demonstrated that the washing had been complete. Activity of the enzyme in a washed suspension of intact tumor cells was determined at the same time.

Tyrosine aminotransferase activity was determined from the increase in p-hydroxyphenylpyruvate [1], and glucose-6-phosphatase activity was determined from the accumulation of inorganic phosphate by a method modified by the authors [7]. Glucose-6-phosphatase in the cells was demonstrated histochemically by Gomori's method [4]. Glucose oxidase activity was determined in 2 ml of a mixture containing (final concentration): 0.25 M acetate buffer, pH 4.8; glucose 0.05 M; o-toluidine 0.0012 M; NaCl 0.15 M; peroxidase 0.05 mg, and expressed in micromoles of converted glucose. The same mixture was used for the cytochemical detection of glucose oxidase in single cells.

Central Scientific-Research Laboratory and Department of Biochemistry, Moscow Medical Stomatological Institute. Department of Biochemistry, P. Lumumba People's Friendship University, Moscow.
(Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Orekhovich.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 73, No. 2, pp. 92-94, February, 1972. Original article submitted May 14, 1971.

TABLE 1. Activity of Foreign Enzymes in Residues of Ehrlich's Carcinoma Cells (in nmoles product or substrate/min per ml residue at 37°C)

Group of animals	Tyrosine aminotransferase		Glucose-6-phosphatase		Glucose oxidase	
	n	$M \pm m$	n	$M \pm m$	n	$M \pm m$
Experimental (enzyme injected in vivo)	9	15,12±2,18	10	271,29±72,01	6	1 531±1 025
Intact	9	0,81±0,08	7	17±2,54	5	0

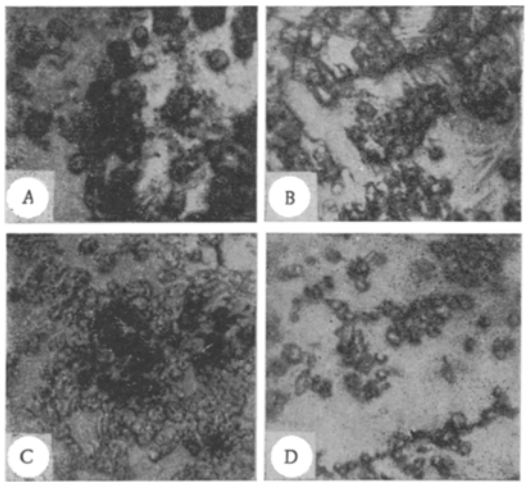

Fig. 1. Glucose oxidase (A and B) and glucose-6-phosphatase (C and D) in washed Ehrlich's carcinoma cells. A, B) cells from mice receiving enzyme preparations by intraperitoneal injection; B, D) cells of intact mice.

EXPERIMENTAL RESULTS

The experimental results are given in Table 1.

The activity of the injected enzymes in intact Ehrlich's carcinoma cells is minimal or absent, which was why they were choosen as test objects. In all the experiments fairly high activity of these enzymes was found in the residues of cancer cells, washed with physiological saline, obtained from animals receiving injections of the enzyme preparation. No activity of the enzymes was found in the last portions of the washing liquid; it was present only in the cell residue. The experiments with glucose oxidase (which is absent from all animal tissues, including those of tumors) showed that activity of the complexes is due to "binding" of the enzymes with the tumor cells, and not to activation of traces of these enzymes present in the intact tumors. The appearance of a large number of cells possessing foreign enzyme activity was clearly demonstrated by histochemical methods (Fig. 1).

A study of the histochemical preparations of cells with glucose-6-phosphatase activity showed that besides single uniformly stained cells there were others in which there were concentrations of the enzyme at the surface and dense collections of darkly stained cells. The binding of tumor cells (washed) with enzymes added to the medium was also observed in experiments in vitro when the suspension was agitated on a shaker for 2 h at 37°C.

Complexes of cells with tyrosine aminotransferase were resistant (retained their activity) during incubation for 20 h in nutrient medium at 37°. Many more nonviable cells (stained with trypan blue) were observed in samples treated with liver enzymes than in the intact suspensions before incubation.

The nature of the tumor cell–foreign enzyme complex has not yet been explained; one assumption is that the enzyme penetrates inside the cell, as has been demonstrated for nucleic acids [2, 5] and ribonuclease [6, 8]. However, the possibility of adsorption of the enzyme protein on the surface of the tumor cells and of more complex processes of interaction between the enzymes and the cell structures cannot be ruled out.

The ability of tumor cells to take up large quantities of foreign enzymes from the surrounding medium suggests that this phenomenon may also take place during growth of a spontaneous tumor, when the donors of the enzymes can be the surrounding tissues or blood. It is all the more probable that enzyme preparations, when administered as antitumor agents, can be bound by large numbers of tumor cells.

LITERATURE CITED

1. F. B. Levin, Vopr. Med. Khimii, No. 3, 315 (1969).
2. V. P. Tomsons and M. A. Pirozhkova, Izvest. Sibirsk. Otdel. Akad. Nauk SSSR, 1, No. 5, 108 (1970).
3. A. Ando and K. Chisada, Radioisotopes, 19, 276 (1970).
4. M. Burstone, Enzyme Histochemistry [Russian translation], Moscow (1965).
5. L. Gibb and E. Kay, J. Cell Biol., 38, 452 (1968).

6. A. Hakim, Exp. Med. Surg., 14, 202 (1956).
7. A. Harper and E. Young, Biochem. J., 71, 696 (1959).
8. L. Ledoux, Biochem. J., 66, 25 (1957).