CHANGES IN PERMEABILITY OF CELL MEMBRANES AND IN COMPOSITION AND PROPERTIES OF HEXOKINASE DURING INDUCED CARCINOGENESIS

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The isozyme spectrum of hexokinase (HK) of the mucous membrane of the rat large intestine consists of three isozymes. In tumors induced by 1,2-dimethylhydrazine in this situation, the isozyme composition of HK is simplified through the loss or sharp reduction in content of the isozyme with the fastest speed of migration toward the anode. In metastatic tumors HK is represented by a single isozyme. The Michaelis constant (glucose) for KG in the tumors is reduced by 2-3 times. Starting from the first month of administration of the carcinogen, HK activity begins to appear in the blood serum of the animals and by the fifth month it is found in 80% of cases. HK was always absent from the blood serum of control animals.

KEY WORDS: carcinogenesis; intestine; hexokinase and its isozymes; blood serum.

The study of the biochemical characteristics of transplantable and induced tumors of animals has revealed certain of the properties of a "tumor" hexokinase (HK) that distinguish this enzyme from its normal prototype. Besides simplification of the isozyme composition of the "tumor" HK, the total HK activity is sharply increased [17, 18], and the kinetic parameters of the isolated HK are modified [5]. These characteristic features of "tumor" HK have also been observed during the investigation of malignant neoplasms in man [4, 13]. Elimination of enzymes of the glycolytic cycle into the body fluids of the tumor-bearing animal has been demonstrated [1, 15, 16]. The appearance of HK activity in the blood serum in patients with malignant neoplasms and its absence in the serum of healthy subjects reflect a particular stage of tumor development and can be used as a diagnostic test for the disease [2, 3]. However, no investigations of the dynamics of changes in the composition and properties of HK and of the passage of this enzyme into the circulation at different stages of carcinogenesis have yet been undertaken.

The object of this investigation was to study the isozyme composition and kinetic properties of HK and the permeability of cell membranes for this enzyme in the course of induced carcinogenesis.

EXPERIMENTAL METHOD

Tumors of the intestine induced in rats by weekly subcutaneous injections of 1,2-dimethylhydrazine (DMH) in a dose of 21 mg/kg body weight were investigated. In their histological structure the tumors were chiefly adenocarcinomas and mucoid and spheroidal-cell carcinomas. Visually the tumors were detected 4-5 months after the beginning of injection of the carcinogen. The spheroidal-cell carcinomas gave metastases toward the end of the fifth month. Important features distinguishing the chosen model were the 100% induction rate of tumors, their localization predominantly in a particular segment of the intestine, and their synchronized appearance in all the experimental animals [6]. Healthy intact animals were used as the control.

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TABLE 1. Changes in K_m (for glucose) of HK of Mucous Membrane of Descending Colon (DC) and Tumors in that Situation Depending on Duration of DMH Injection

Test object	Duration of injection of carcinogen (in months)	Number of experiments	K_m (glucose), in moles · 10^{-5}
Normal mucous membrane of DC	–	10	1.35
Mucous membrane of DC	1	10	1.50
" " "	2	10	1.62
" " "	3	10	1.54
Total tumor tissue	4	10	1.25
Adenocarcinoma	5	8	1.00
Spheroidal-cell and mucoid carcinoma	5	9	0.82
Adenocarcinoma	6	9	1.23
Spheroidal-cell and mucoid carcinoma	6	7	0.65
Metastases of spheroidal-cell carcinoma	6	6	0.58

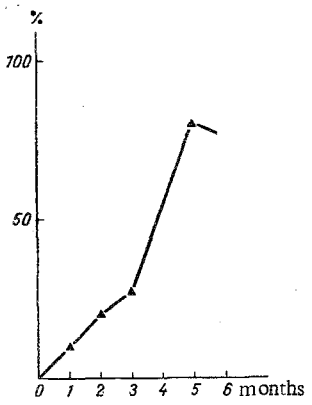

Fig. 1. Frequency of detection of hexokinase activity in blood serum of rats versus duration of DMH injection. Abscissa, duration of injection of DMH (in months); ordinate, frequency of detection of HK (in %).

The isozyme composition and catalytic properties of HK were investigated in the soluble fraction of homogenates of the mucous membrane of the descending colon, where tumors arose in virtually all the experimental animals, and in the developing tumors. To prepare 50% homogenates, the separated mucous membrane or tumor was homogenized in a mixture of the following composition (in M): sucrose 0.25, tris-HCl 0.005, 2-mercaptoethanol 0.0025, EDTA 0.002; pH 7.4. The soluble fraction was separated by ultracentrifugation for 2.5 h at 105,000 g. The protein content in the fraction was determined by Lowry's method [4]. HK activity was measured spectrophotometrically [9]. Disc electrophoresis for investigation of the HK isozyme spectra was carried out in 7.5% polyacrylamide gel as described by Davis [10]. Staining for specific HK activity in the gel was carried out by Eaton's method [11]. The Michaelis constant (K_m) for glucose was determined by the Lineweaver–Burke method. Tests were carried out 1, 2, 3, 4, 5, and 6 months after the beginning of DMH administration. HK activity of the blood serum was studied at the same time. Ten to 15 animals were used in each experiment.

EXPERIMENTAL RESULTS AND DISCUSSION

As Fig. 1 shows, starting from the 2nd-3rd month of injection of the carcinogen, HK activity was found in the serum of about one-third of the experimental animals. The frequency of appearance of HK in the serum increased as the tumor developed progressively and reached 80% by the fifth month, when multiple tumors were present in the intestines of all the rats. It is important to note that HK activity was found in the serum at a time when only the initial neoplastic changes could be found microscopically in the mucous membrane of the large intestine, with the appearance of small groups of atypical cells and foci of carcinoma in situ. No HK activity was found in the serum of any of the control animals.

The results of determination of K_m (for glucose) of HK from the soluble fraction of homogenate of the mucous membrane of the colon and tumors of this region are given in Table 1. Starting from the fourth month a tendency can be seen for the value of K_m to decrease. Possible K_m started to decrease a little earlier, but the tumor could be detected macroscopically and it could be separated only from that time. The greatest changes in the value of K_m were observed in spheroidal-cell and mucoid carcinoma. The affinity of HK for glucose in tumors with that histological structure and also in the metastases of the spheroidal-cell carcinoma was more than doubled.

The study of the HK isozyme spectrum of the normal mucous membrane of the descending colon showed that it consists of three isozymes (Fig. 2a). Other workers have also found a similar distribution of isozymes in the rat intestine [12, 19]. In spheroidal-cell and mucoid carcinoma (Fig. 2c) the isozyme migrating fastest toward the anode disappeared from the spectrum. An even simpler pattern was observed in tests

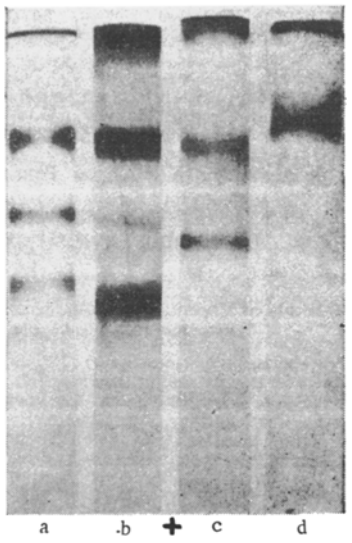

Fig. 2. HK isozyme spectra of normal mucous membrane of descending colon and malignant tumors of that region: a) normal mucous membrane; b) adenocarcinoma; c) spheroidal-cell carcinoma; d) metastases of spheroidal-cell carcinoma. Direction of movement toward anode.

of metastatic foci of colloid carcinomas (Fig. 2d). In addition to inhibition of two isozymes, the content of the "slowest" HK isozyme was sharply increased. A somewhat different picture, with partial inhibition of the second isozyme, was found during investigation of the isozyme spectrum of HK from the adenocarcinoma (Fig. 2b).

These investigations confirm that the study of the properties of "tumor" HK can serve as a definite biochemical criterion of the progressiveness of tumor growth. The appearance of HK in the blood serum only one month after the beginning of injection of the carcinogen indicates a disturbance of permeability of the cell membranes of the transformed cells in the very earliest stages of carcinogenesis, i.e., when the tumor could not yet be detected macroscopically. Changes in function of the genome during malignant transformation are also reflected in the properties of the HK itself. HK from tumor tissue has a higher affinity for glucose (a decrease in the value of K_m) than that from the normal homologous tissue and its isozyme spectrum is different. On the other hand, it will be noted that tumors with different histological structure are characterized by specific features of their HK isozyme spectrum: the HK isozyme spectrum of adenocarcinoma differs from that of spheroidal-cell and mucoid carcinoma and by an even greater degree from the isozyme spectrum of HK from metastatic foci. This conclusion agrees with the results of the study of HK from normal and malignant human tissues, when differences were found in the HK isozyme spectra obtained from different sources [4].

Changes in membrane permeability and in the properties of HK are presumably the results of the direct action of the carcinogen on cells of the intestinal mucous membrane. However, the study of the kinetic properties and isozyme composition of HK in the soluble fraction of homogenates from the mucous membrane of the ileum, where tumors never arise following administration of DMH, disproves this hypothesis. Parallel investigations showed that throughout the period of administration of DMH the isozyme spectrum and K_m values of HK in the mucous membrane of the ileum remained unchanged.

The results of the present experiments, showing changes in the properties of HK in the early stages of induced carcinogenesis, agree with results obtained by other workers who found a sharp increase in HK activity in mouse skin tumors induced by benzpyrene [8] and in epidermal hyperplasia resulting from the action of 7,12-dimethylbenz-α-anthracene, when the HK activity in the target tissue increased from the first week after the beginning of administration of the carcinogen [7].

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