HISTOCHEMICAL STUDY OF OXIDO-REDUCTIVE ENZYMES DURING VIRUS CARCINOGENESIS

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Neoplastic growth induced in the subcutaneous cellular tissue of hamsters by monkey adenovirus SA7 (C8) is accompanied by changes in the state of oxido-reductive enzymes both in cells of fibroblast type and in the tumor cells. These changes can be conventionally divided into two stages. In the first stage, covering the first 3 days, activity of these enzymes was depressed. The next two stages were associated with changes in the shape of the diformazan deposits in the cells, probably as a result of changes in the mitochondrial membranes. The changes in the activity of these enzymes observed in the second and, in particular, in the third stages are characteristic of the biochemistry of tumor cells regardless of the type of carcinogenesis.

During carcinogenesis changes take place in energy metabolism and, in particular, respiration is disturbed [3]. One way of investigating these problems is to use histochemical methods of detecting oxido-reductive enzymes. There are many reports in the literature of such investigations of human tumors and tumors produced by experimental chemical carcinogenesis [5, 6, 9, 11]. However, insufficient work has been done on virus carcinogenesis, and this has mainly dealt with tumors induced by Rous sarcoma virus.

This paper describes a histochemical study of the state of oxido-reductive enzymes concerned with the principal stages of glycolysis, the Krebs' cycle, and electron transport. Tumors induced in the subcutaneous cellular tissue of hamsters by monkey adenovirus SA7 (C8) was used as the experimental model.

EXPERIMENTAL METHOD

Experiments were carried out on 158 newborn hamsters inoculated subcutaneously with 0.2 ml of the small-plaque variant of undiluted virus in a dose 10^7 PFU/ml. This dose of virus is adequate to induce tumors in 100% of animals [7]. Newborn animals receiving the same volume of the nononcogenic monkey adenovirus SV15(M4) or of medium no. 199 were used as the control. The hamsters were killed between the 1st and 38th days after injection. Until the 20th day inclusive, film preparations were made from the subcutaneous cellular tissue of the animals. Later, sections of the tumors were cut in a cryostat. Both in the films in the sections the state of the following oxido-reductive enzymes was demonstrated: NAD- and NADP-diaphorases, succinate dehydrogenase (SDH), and glucose-6-phosphate, α-glycerophosphate, lactate, glutamate, and isocitrate dehydrogenases. The films and sections of the tumors were stained simultaneously with Yasvoin's iron-hematoxylin and with hematoxylin-eosin.

EXPERIMENTAL RESULTS

From the 7th day, solitary cells with unusually large, round or oval nuclei were found in the subcutaneous tissue of the hamsters infected with SA7(C8) virus. On the 10th-12th day the first foci of proliferation, consisting of cells of the same type but now polymorphic in character, were found. Other foci of proliferation, which were sometimes found in the same specimens, consisted of spindle cells. The

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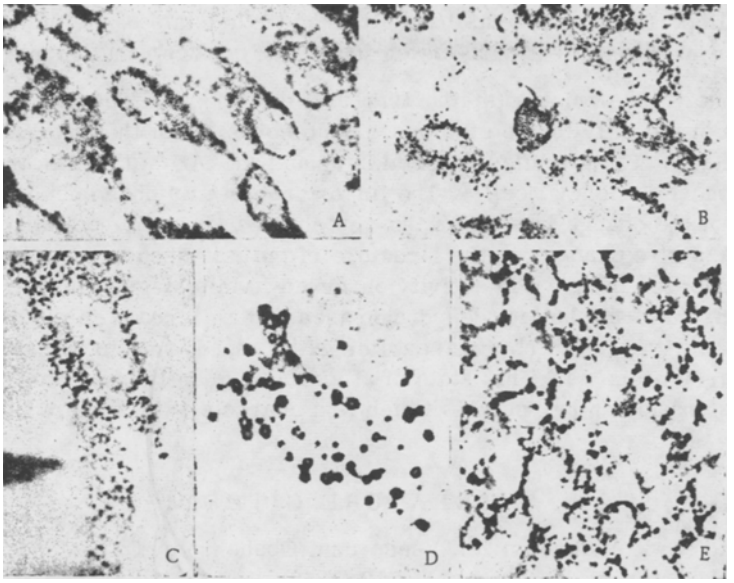

Fig. 1. Neoplastic growth and changes in state of oxido-reductive enzymes at various stage of formation of tumors induced by monkey adenobirus SA7(C8). A) NAD-diaphorase in fibroblasts 24 h after injection of medium 199, 345 ×; B) decrease in NAD-diaphorase activity on 3rd day after infection with SA7(C8) virus., 345 ×; C) NAD-diaphorase in a fibroblast 24 h after infection with SA7(C8) virus. Immersion; D) change in shape of diformazan deposits in cells on 5th day after infection with SA7(C8) virus. NAD-diaphorase, immersion; E) monomorphism of diformazan granules in tumor. NAD-diaphorase, immersion.

tumors which developed (after the 19th-20th day) were relatively undifferentiated sarcomas. Some tumors consisted of fairly monomorphic cells with large nuclei and scanty cytoplasm, while others, or parts of the same tumors, consisted of more polymorphic epithelioid cells. Areas formed by spindle cells showing a tendency to be grouped in bundles were usually present. Numerous thin-walled blood vessels were present in the tumors. The structure of these tumors is described in the literature [2].

The changes in the oxido-reductive enzymes in the cells of the subcutaneous tissue of the hamsters observed after injection of the SA7(C8) virus can be divided conventionally into three stages. The first stage covers the first 3 days. No significant difference was noticed in the stage of the enzymes in the animals inoculated with SA7(C8) compared with the control 24 h after infection (Fig. 1A). However, after 3 days considerable inhibition of the activity of all these enzymes was found in cells of fibroblast type in the experimental group of hamsters (Fig. 1B). In some cells diffuse deposits of diformazan were found along with granules, a picture most clearly seen in sections stained for diaphorases. According to some workers [10], this is due to ergoplasmatic NAD- and NADP-diaphorases and it may reflect changed relationships between the cell organoids participating in metabolism.

The second stage of the changes in the state of the enzymes covered the period from the 5th to the 20th days, i.e., until the appearance of fully formed tumors. Enzymic activity after 5 days was much higher than in the previous period and reached its initial level (malate and glutamate dehydrogenases) or actually exceeded it (glucose-6-phosphate and lactate dehydrogenases). Meanwhile the activity of succinate, isocitrate, and α-glycerophosphate dehydrogenases remained somewhat below normal. In the same period solitary cells with a considerably modified type of diformazan deposits were found (Fig. 1C, D). Foci of proliferating tumor cells appearing on the 10th-12th days consisted of cells with a similar modified type of diformazan deposits in a narrow rim of cytoplasm. On the 14-19th days the same diformazan deposits were also observed in spindle cells, forming a number of foci of proliferation. The changes in shape of the diformazan deposits may correspond to a disturbance of permeability of the mitochondrial membranes [4].

In the light of the great importance which has been attached to the state of the membranes during malignant change [1, 8], this change in permeability may be an important factor in malignant transformation.

The third stage covers the period after the 19th-21st day and until death of the animals and it reflects the state of the enzymes in the fully formed tumors. Monomorphism of the diformazan deposits in the cells was characteristic (Fig. 1E). The granules differed in appearance from those in the previous stages, but they also differed from those in normal cells. The importance of this phenomenon is not adequately clear because the mechanism of the change in state of the mitochondria in tumor cells has still received comparatively little study. Adaptive changes in the structure of the mitochondria, for example, may be conjectured. The subsequent changes in enzymic activity observed in this stage, with a resulting increase in the activity of glusoce-6-phosphate and lactate dehydrogenases and a decrease in the activity of NAD. and NADP-diaphorases, agree with results obtained earlier with respect both to human tumors and to various types of experimental carcinogenesis. This state of affairs is evidently connected with a change in the relationship between respiration and glycolysis which is a characteristic feature of the biochemistry of the tumor cell [12].

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