COMPARATIVE CYTOCHEMICAL STUDY OF SUCCINIC DEHYDROGENASE AND ALKALINE PHOSPHATASE ACTIVITY IN PRIMARILY EXPLANTED AND TRANSPLANTABLE TISSUE CULTURE CELLS

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It was previously shown that during the first three days of culturing of first generation renal cells alkaline phosphatase [2] and succinic dehydrogenase [3] activity in these cells markedly decreases. On the other hand, NEr-2 strain cells exhibited a considerable succinic dehydrogenase activity at all times. It was noted that endogenous dehydrogenase activity was relatively high in the first generation renal cells and low in NEr-2 strain cells [3]. However, it seemed advisable to check this previously received data on other tissue cultures, including primarily explanted and transplantable cells of the same origin. The literature pertinent to the question is cited [2, 3].

EXPERIMENTAL METHOD

The cells studied included NEr-2 strain, heart cells of a monkey [Macacca cynomolgus (SOZ)], human amnionic epithelial cells,* first generation amnionic cells and second generation Macacca rhesus renal cells. The methods of culturing cells and determining enzymes were described previously [2, 3]. The cells were removed from the glass surface with 0.03% versene and, in some experiments with SOZ cells, with 0.25% trypsin. The enzymatic activity was studied during 9 to 10 days of culturing, in the cell suspension immediately following treatment with trypsin (or versene) and in frozen sections of amnionic membranes. Each tissue culture was subjected to three series of experiments. Trypsin treatments of SOZ cells were repeated twice.

EXPERIMENTAL RESULTS

Alkaline phosphatase. Alkaline phosphatase activity was relatively low in the freshly frozen epithelial cells of the amniotic membrane and in the cell suspension immediately following treatment with trypsin. Cobalt sulfide precipitate, according to which the distribution and presence of enzyme was judged, was almost entirely absent during the first three days of culturing. In the ensuing days the amount of precipitate increased. In general the alkaline phosphatase activity level in the cells of amniotic membrane, cell suspension, and culture was relatively low.

The incubation time for determining succinic dehydrogenase in the tissue culture cells was shortened from 2 hours to 1 hour and even to 30 minutes as compared to the previously reported technique [3]. This was done in order to obtain better localization of enzyme activity in the cells of transplantable strains.

* The strain of these cells was obtained at the Moscow Research Institute for Antipoliomyelitis Preparations by T. G. Orlova and was kindly provided by her for our study. Treatment with trypsin and culturing the first generation amniotic cells were also performed by T. G. Orlova, for which we are extremely grateful to her.

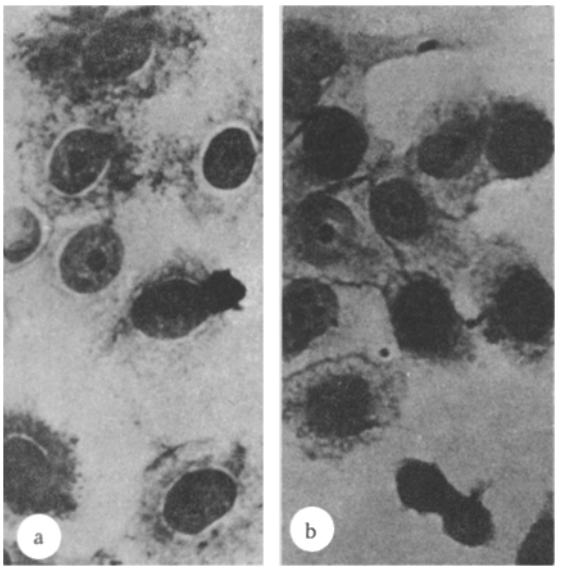

Fig. 1. Alkaline phosphatase activity in transplantable cells. First day of culturing. a) Amniotic cells treated with versene; b) SOZ cells treated with trypsin. Photomicrographs × 500.

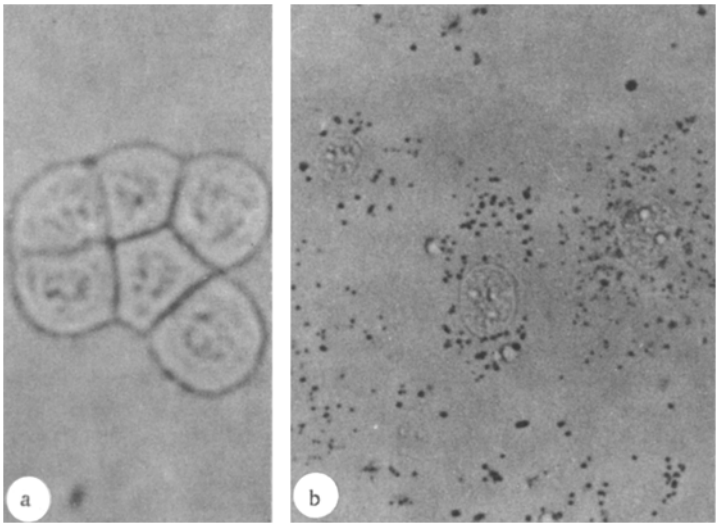

Fig. 2. Succinic dehydrogenase activity in primarily explanted amniotic cells. a) First day of culturing; b) seventh day of culturing. Photomicrographs × 500.

In studying renal tissue subculture it was found that cells in suspension as well as cells in culture had a very low alkaline phosphatase activity level. It was noted that the second generation of renal tissue culture was composed of "spindle" and "flat" cells, while the first generation of renal tissue culture cells showed enzyme-containing "nest" cells [2]. However, it should be noted that it was possible to find individual cells with high alkaline phosphatase activity in a few preparations.

In distinction from primarily explained cultures, the cells of all the transplantable strains examined posessed high alkaline phosphatase activity. The intensity of reaction during the first few days of culturing

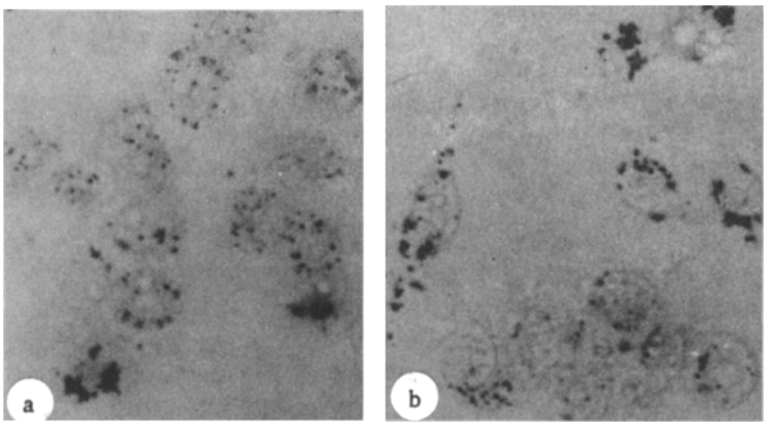

Fig. 3. Succinic dehydrogenase activity in transplantable cells. a) Amniotic cells, first day of culturing, treated with versene; b) amniotic cells, 6th day of culturing. Photomicrographs x 500.

was only slightly lower than in the cell suspension immediately following treatment with versene (Fig. 1a). The treatment with trypsin solution, as it was performed in two series with SOZ cells, did not lead to a decrease in enzyme activity (Fig. 1b). It was of interest to note that in transplantable cells of human amniotic epithelium the intensity of reaction was just as high as it was in other transplantable cell strains, despite the fact that enzymatic activity was very low in primarily explanted amniotic cells. It must be noted that while the general content of cobalt sulfide precipitate was high in the transplantable cells, there also were a few cells with relatively low intensity of enzymatic reaction.

Succinic dehydrogenase. Moderate deposits of diformazan granules were found in cells of unfixed frozen sections of human amnionic membrane. Cells in suspension, immediately following treatment with trypsin, likewise contained a moderate precipitate. The intensity of reaction in some cells was minimal. A striking decrease in enzymatic activity occurred during the first 2 to 3 days of culturing (Fig. 2a). Only on the 4th day did some cells show diformazan granules. The intensity of reaction increased up to 6 to 7 days (Fig. 2b), stabilized at this level and began to decrease from the 10th to 11th days. The endogenous activity of dehydrogenases in the cells of the 3 to 7 day culture was well expressed, and its level was only slightly lower than that of succinic dehydrogenase activity.

The dynamics of enzymatic activity changes were uniform in all transplantable cell strains studied. The cell suspensions from these cultures were extremely rich in needle-like crystals of diformazan. The reaction was absent only in isolated cells and could be explained by nonviability of these cells. Likewise endogenous dehydrogenase activity level was relatively high in cells from suspensions. During the 1st day of culturing, despite some decrease, the activity of succinic dehydrogenase was still high (Fig. 3a). By the 2nd day the intensity reached a very high level, and maintained it up to the 7th and 8th days.

Diformazan precipitate which was formed in transplantable amnionic cells was more delicate than in other transplantable cultures. This could be probably explained by the "young" age of the cell strain, which was started in March, 1959. Endogenous dehydrogenase activity in all transplantable strains was low during the entire duration of culturing. Intensity of the reaction in trypsin-treated SOZ cells was equal to that found in cells removed from the glass by conventional methods (Fig. 3b).

Succinic dehydrogenase activity in the 2nd generation cell suspension of renal tissue was relatively high. Only a few individual cells did not contain a precipitate. It was noted that during the 1st day of culturing the subculture cells showed only an insignificant decrease in enzymatic activity. However, by the second day the activity began to increase and remained at this relatively high level up to the end of culturing. The endogenous activity level of dehydrogenases in the cell suspension was considerable during the first 5 to 6 days, then began to decrease somewhat. The granules of diformazan precipitate in the cells of subculture were small, and attained larger size only on aging.

It was proposed that a reversible decrease in alkaline phosphatase and succinic dehydrogenase activity in the early phases of 1st generation renal cell culture and an absence of such a decrease in the cells of NEr-2 strain could be explained by adaptation of cells to conditions of tissue culture [2, 3]. These observations were confirmed in the present study on a number of different tissue culture strains. The results showed that changes in activity of the enzymes studied were similar in various tissue culture strains. Dynamics of enzyme activity in these cultures corresponded to those of mitotic activity. During the first days of culturing of monkey renal tissue cells and human amnion the mitotic index was 1 to 2‰. Only in the latter stages did it reach 25-35‰ [1]. In the transplantable cell strains it already reached 50 to 70‰ during the first two days of culturing [1].

The fact, first reported by L. G. Stepanova [4], that only a small percent (7 to 8%) of primarily explanted cells was attached to glass while transplantable strains showed 95 to 100% attachment could be explained by various degrees in adaptation. A sharp decline in enzymatic activity and in mitotic index during the early stages of culturing of primarily explanted tissues could be explained by a specific action of trypsin, used in obtaining these cultures. However, absence of any discrepancies in parallel experiments employing trypsin and versene on SOZ cells spoke against it. Numerous reports of studies employing trypsin solution in making passages of transplantable strains likewise did not mention retardation in cellular growth [6, 8, 9].

It was previously reported [5, 10, 11] that transplantable liver cells of Chang's strain, when compared with parenchymatous liver cells, lacked several enzymes. This phenomenon was explained on the basis of cellular dedifferentiation and primitive metabolism in the tissue culture environment. In this connection our findings of sharp rise in alkaline phosphatase and succinic dehydrogenase activities in transplantable amniotic cells as compared to a culture of primarily explained amnion are of particular interest. It is possible that during the process of cellular adaptation to tissue culture environment, concomitantly with disappearance of some enzymatic system activities there occurs a sharp rise in others.

Reported factual material completely confirms the data of Barlington [7], who found a considerable decrease in activity of a number of enzyme systems in cells of primarily explanted renal tissue in comparison with the same cells in suspension. This author, as well as we, explained changes in enzyme activity by an altered environment existing in vitro. Present data concerning endogenous dehydrogenase activity in various tissue cultures confirms previously obtained results [3].

Subcultured renal tissue cells occupy an intermediate position between primarily explanted and transplantable tissue cultures. Endogenous dehydrogenase activity in these cells is relatively high, and in this respect they resemble primarily explanted cultures. On the other hand, these cells did not show a sharp decline in enzyme activity, which suggested similarity to transplantable cultures.

SUMMARY

The activity of succindehydrogenase and of alkaline phosphatase was studied in the transplantable strains of amniotic epithelium, NEr-2 and the heart of Macacca cynomolgus, as well as in the first generation cells of amnion and the second generation cells of the monkey kidney. In difference from the primarily explanted amniotic cells, the activity of the enzymes does not disappear from the cells of transplantable strains and the second generation of the monkey kidney. The endogenous activity of dehydrogenase was relatively high in the primarily explanted amniotic cells and the cells of the second generation of monkey kidney tissue. A possibility of connection between the changes of the enzyme activity and the processes of cellular adaptation to the conditions of culturing is discussed.

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